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THE STAMINATE CONE AND MALE GAMETOPHYTE OF PODOCARPUS

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(WITH NINE FIGURES AND PLATES VIII AND IX)

Knowledge of the gametophytes of the Podocarpineae is at the present time limited to that contained in three brief papers. In 1902 COKER (**3**) published an account of two species of Podocarpus; in June 1907 JEFFREY and CHRYSLER (**5**) added some new facts concerning Podocarpus and Dacrydium; and in September of the same year Miss YOUNG (**14**) gave an excellent résumé of the subject of the male gametophyte and added several new and interesting details for Dacrydium. COKER records that in *Podocarpus coriacea* there are produced two primary prothallial cells, both of which may divide amitotically; a tube nucleus; and a primary spermatogenous cell, which gives rise to a stalk cell and a body cell. The body cell undergoes nuclear division and one of the nuclei is then extruded from the cytoplasm, leaving a single functional male cell. JEFFREY and CHRYSLER add to this the interesting details that from the two primary prothallial cells there may arise by subsequent division as many as eight prothallial cells. Of still greater interest is the record of the "proliferation" of the primary spermatogenous cell, whereby there arise three cells placed transversely, the middle one of which is considerably larger than either of the others. Miss YOUNG reports that in Dacrydium one or both of the primary prothallial cells may divide once. From the primary spermatogenous cell arise two cells, one of which is usually sterile, the other of which functions as the body cell. The interesting observation is made that there is a

tendency for both derivatives of the primary spermatogenous cell to function as body cells.

The material for the present study was secured through the kindness of Dr. L. COCKAYNE of Christchurch, New Zealand, and Miss INEZ FRANCES STEBBINS of the Huguenot College at Wellington, Cape Colony, South Africa. To both are due our thanks for their kindness in obtaining the material at no inconsiderable personal inconvenience. Though the collections were necessarily few, yet they include a considerable range of developmental stages in each of the species. The material was fixed in a 5 per cent. solution of commercial formalin in 70 per cent. alcohol. The fixation has proved unexpectedly good, except for stages from the rounding-up of the mother cells to the time when the microspores have a good firm wall and abundant contents. The shrinkage and general distortion within the limits just mentioned have rendered necessary the omission of a number of what appear to be rather interesting and perhaps important details. Some of these will be mentioned briefly in the general account. It is hoped that other material so killed and fixed as to show details of cytological structure will be in hand within the near future. The preparations have been imbedded in 54° paraffin, cut 3–5 μ, and stained in Haidenhain's iron alum hematoxylin stain, alone or counterstained with various aqueous stains such as orange G, Bismarck brown, erythrosin, etc., and with the saffranin gentian-violet combination. Three species have been examined, though not covering exactly the same ground.

Observations

The cones of *P. totarra Hallii* are of a generally oval shape, varying from 3 or 4 to 15 or 20^{mm} in length, and from 2 to 4^{mm} in diameter. They are usually in pairs, one being large and one small. The base of the cones is surrounded by several closely appressed, rigid, scalelike bracts. The peduncle is quite short or almost lacking. The cones of *P. nivalis* are somewhat shorter, not paired, and decidedly slenderer. The cones of the third species (the label of which was lost in transit) are similar in shape to the first ones, but are smaller. In other respects the cones are very similar. *P. totarra* is one of the tallest of forest trees and *P. nivalis* a very small

shrub, sometimes creeping. The third species may be *P. elongata*, a common forest tree of the South African region. This is a mere inference from its abundance in the region from which this material

FIG. A.—Group of *P. dacrydioides* in remnant of taxad forest, N. Z.—Photograph by J. CROSBY SMITH.

was received, and from the fact that I have received other material from the same source belonging to this species.[1]

In cross-section a staminate cone shows about ten to twelve sporophylls, each with two sporangial cavities (*fig. 1*). The vascular system seems to be rather weakly developed, but shows a distinct endarch collateral bundle (*fig. 3*) corresponding to each sporophyll. Just outside of the bundle there usually is found a resin canal (*fig. 1*).

FIG. B.—Buttressed base of *P. dacrydioides*, in ancient forest of Canterbury Plain, N. Z.—Photograph by L. COCKAYNE.

The resin canals contain little material of any sort in the preparations available for study. The distinctly glandular cells lining them (*fig. 2*) suggest, however, that they are functional. The number varies in different strobili, but in those examined did not fall below six nor exceed eighteen or twenty.

The sporophylls as seen from the upper surface are somewhat spatulate, with a rather pointed apex. *Fig. 4* presents an outline

[1] We have received from Dr. COCKAYNE two excellent photographs of *P. dacrydioides*, which are reproduced on account of their general interest (*figs. A* and *B*).

of a longitudinal section somewhat to one side of the middle, and will give an idea of the side view, as well as the relation of the sporangial cavity to the sterile portion of the leaf. *Fig. 6* shows a cross-section of a sporophyll near the middle of the sporangium. The sporangia are somewhat less prominently exposed on the under side than those of Pinus, for example. A single weakly defined vascular bundle (*fig. 6*) traverses the upper part of the central sterile septum and is sometimes accompanied by a resin canal lying below it and between the sporangia. The wall of the sporangium varies in thickness somewhat, but in general on the freely exposed part it is about four or five cells thick (*fig. 5*). One or two of the inner layers are slightly differentiated as a tapetum. This is not very evident and does not occur until the sporogenous tissue has nearly reached the mother cell stage. The tapetum does not long persist, but disappears as a functional tissue about the time the young spores have formed a thick wall and wings. All of the wall proper, except the outermost layer of thickened cells, may break down before the spores are shed.

The earliest stages of the sporogenous tissue observed (in *P.* sp.) lacked one or two divisions of the mother cell stage. *Fig. 7* will serve as a typical representative cell of the sporogenous tissue at this stage. The walls are thin and delicate, as is usually the case, and the cytoplasm stains somewhat more densely than that of the wall cells. It was possible to secure very good preparations of this, considering the nature of the killing and fixing agent. The cells were slightly shrunken, so as to have pulled away in many places from one another or from their own walls. *Fig. 7* will show the principal facts. The cytoplasm does not seem to present either a typically reticulated or a foam structure, but rather resembles a sort of flocculent precipitate scattered more or less irregularly through the cell. These flocculent masses may assume a sort of feathery, filamentous, more or less branched form, or they may appear as scattered masses of irregular shape. The filaments and other masses do not seem to be connected in any definite manner, but to be distributed through the cell by chance. No stored food can be detected microscopically in the cytoplasm at this time, though the parenchymatous cells of the axial portion of the strobilus contain considerable quantities of large

starch grains. The wall cells of the sporangium do not at this time, as in some sporangia (Ophioglossum, for example), contain starch, but are relatively poor in cytoplasm and cytoplasmic inclusions. There is relatively little change in the cytoplasm of the sporogenous tissue as growth advances, except that it grows gradually less dense until at the time of the formation of the young spores there is almost no stainable substance left.

The nuclei of the sporogenous cells (*fig. 7*) previous to the mother cell stage show a structure that is very like that of the cytoplasm just described, except that it is denser and there is more of a tendency to aggregate itself into disconnected masses. There are one or more large nucleoli present in the nucleus at this stage, as there seem to be in the resting nuclei of all the stages of all the tissues examined.

As the nucleus approaches division, the nuclear substance begins gradually to arrange itself into filaments or strands (*fig. 8*) which may end freely or anastomose with other filaments or masses. About the same time the hitherto nearly uniformly staining material begins to stain differentially, so that filaments seem to link together knots of more deeply staining material. Whether this indicates a difference of substance, as some think, or merely a difference in the state of aggregation, as has been recently maintained by several authors, cannot be said with certainty. However, the gradual derivation of this structure from the preceding one, in which everything seems homogeneous, would certainly point to the latter interpretation as the correct one. During this time the nucleolus (or nucleoli) gradually loses its density, as shown by the staining reactions. It continues to do so until it finally does not stain at all or has disappeared entirely. *Fig. 9* shows a surface view of a nucleus in which the filaments have become more regular and somewhat thicker. In *fig. 10* is shown a spirem, into which the filamentous stage gradually passes, in which the deeply staining knots on the spirem are unusually prominent. The figure shows nearly all of the spirem that was included in a 3-μ section; therefore it probably contains one-third to one-half of the entire spirem, since the nucleus is about $7 \times 10\,\mu$. In some cases the spirem consists of darkly stained masses connected by much smaller threads, and in others, as shown in *fig. 10*, by broader strands

which seem to be of different material because of the considerable difference in depth of staining.

The spirem gradually becomes denser and ramifies all through the nucleus (*fig. 11*), the nucleolus disappears, and finally the spirem breaks into elongated loops, which arrange themselves into a tangled mass at the equatorial plate. The exact method of division was not made out owing to the close tangling of the long chromosomes. In the anaphase the chromosomes appear as twenty-four straight elongated rods of about half the length of those entering the mitosis (*fig. 12*).

As the chromosomes approach the poles, the polar ends draw together and the others spread out, so that the daughter nucleus usually has a concavity on the polar side. The chromosomes gradually lose their density and put out processes on one side or the other to fuse with those put out by neighboring ones, thus establishing a sort of reticulate structure (*fig. 13*). This gradually passes back into the stage shown in *fig. 7*. In the reorganizing nucleus one large nucleolus may appear or several small ones, but I did not attempt to discover the origin of them or their relation to one another.

When the cells have reached the mother cell stage, they break loose from one another and round up in the usual manner. As already remarked, the mother cells killed and fixed very poorly, so that only a very meager and perhaps uncertain account of them can be given. About the time the mother cells round up many of them begin to abort; this abortion seemingly may occur as late as the young spores. Up to this time and on to the time of the divisions in the young microspores the development of the sporogenous tissue seems to be nearly simultaneous.

After the mother cells have rounded up, the nucleus soon passes into a condition in which its contents stain so nearly uniformly that it has not proved possible to disentangle its separate constituents. If stained deeply, it appears absolutely uniform, and if the stain be then slowly and gradually drawn, the homogeneous appearance gives way to a sort of pebbled one. Sometimes it looks as if this might be due to the parts of a large and long spirem being crowded closely together. This interpretation is strengthened by the fact that in some cases one can see what looks like the end of a loop pushing out the

nuclear membrane. In other cases the appearance is more that of a great many lumps of some plastic substance thrown into a tight mass. Whether these appearances are normal or not I cannot say. That they represent the only appearances that the present material shows is certain. In the face of the numerous and fairly uniform accounts of sporogenesis in which no such phenomena have been recorded, it is certainly entirely proper at present to ascribe these unusual phenomena to the effects of the reagents. Nothing any more nearly resembling synapsis was seen. It should be noted that of some hundred strobili cut only five showed cells in this stage. However, three of these strobili are the only ones that have shown the reduction divisions. A few sporangia had cells like those shown in *fig. 14*, where the chromosomes are beginning to appear, being almost completely concealed in a mass of granular matter. In the figure the difference in intensity of stain between the chromosomes and the imbedding substance has been greatly exaggerated for the sake of clearness. One gets two views of these developing chromosomes, one in which the chromosomes are mostly seen in cross-section and one in which they lie lengthwise. In some fashion (which the density and abundance of the imbedding substance prevent being made out clearly) out of this comes a rather sharply pointed spindle at whose equator are arranged the short apparently bivalent chromosomes (*fig. 15*). Some preparations seemed to favor the idea that these apparently bivalent chromosomes then split longitudinally. After passing to the poles (*fig. 16*) they became surrounded by a nuclear membrane, but did not, in the few preparations of this stage observed, lose their identity in a resting nucleus. A very few cases of the homotypic mitosis are shown in the preparations and all of these are in the telophase, as shown in *fig. 17*. Walls are not formed till after second division.

Beyond the telophase of the second division the preparations show nothing until after the formation of the wall around the young spores. These are so badly shrunken and distorted that no attempt has been made to figure them. It is observable, however, that the nucleus is at first rather small and oval and lies to one side of the spore cavity, most of which is occupied by an enormous vacuole. *Fig. 18* shows a microspore after the contents have become more abundant. At this

time starch may or may not be present; later it becomes abundant and occurs in relatively large grains (*figs. 30, 31*). The wall has a thick exine much roughened on the outer surface and a distinct intine. The spore coat is usually much thinner on the side away from the point where the prothallial cells will later lie. The external appearance of the pollen grain bears a considerable resemblance to that of Pinus, except that it is smaller, rarely reaching more than 35 μ in the greatest diameter (exclusive of the wings).

The microspore nucleus is large (*fig. 18*) and contains apparently two sorts of material. There is a more deeply staining substance distributed in irregular masses through a very fine ground substance. As the nucleus prepares for division, the amount and density of the more deeply staining substance increases in amount, assumes a more definitely reticulated structure, and finally passes into a rather thick and comparatively short spirem (*fig. 29*). This spirem breaks up into the chromosomes (*fig. 28*), which often still show the distinct segmentation into lighter and darker segments that has already been referred to in discussing the sporogenous tissue. This segmentation is not always found in the chromosomes or even in the spirem. It might be supposed that the different appearance is due to differences in the depth of stain, but this does not seem to be true. The chromosomes are rather long and considerably twisted at the metaphase, but in dividing and passing to the poles they apparently shorten up (*fig. 31*). What has been said of the first division applies equally well to the next, so far as the few mitoses observed show.

One or two prothallial cells are cut off and then the antheridial initial divides to produce the tube nucleus and the primary spermatogenous cell (*fig. 32*). In *P. nivalis* two primary prothallial cells, which do not subsequently divide, seem to be the rule, though one example of the division of the first prothallial was observed. The pollen in the same sporangium varies in the stage of development from microspores to the stage shown in *fig. 32*. These sporangia were, in some cases at least, shedding their spores.

In *P. totarra Hallii* one primary prothallial cell may be cut off, after which the free nucleus divides to form the primary spermatogenous cell and the tube nucleus (*fig. 21*); or two primary prothallial cells may be cut off before the tube nucleus is separated from the primary

spermatogenous cell (*figs. 20, 22*). When only a single primary prothallial cell is cut off, it may (*fig. 19*) or may not (*fig. 21*) divide again. Far more frequently it does divide anticlinally. No case in either species has been observed where either prothallial cell divides periclinally. In case two prothallial cells are cut off, both usually divide anticlinally (*figs. 23, 26, 27*), although the division may be long delayed or fail wholly (*text fig. 4*). The first one may remain undivided, or in rare cases may divide twice, so that the first tier of prothallial

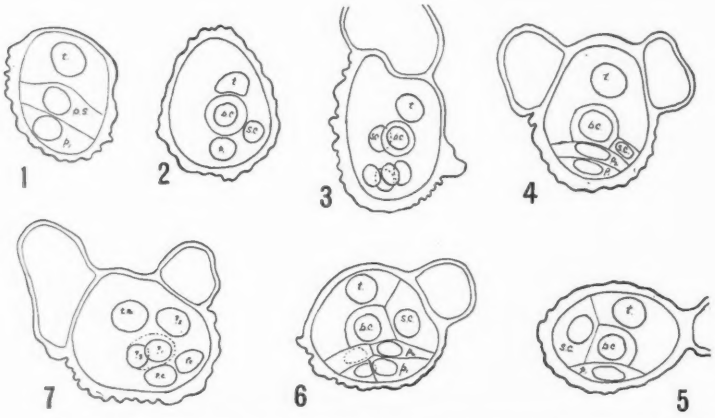

FIG. 1. Transverse section of a male gametophyte consisting of one prothallial cell (p_1), the primary spermatogenous cell (*ps*), and the tube nucleus (*t*).—FIG. 2. Male gametophyte in which the primary spermatogenous cell has divided into the body cell (*bc*) and the stalk cell (*sc*), and in which all the gametophytic walls have broken down.—FIG. 3. Same as the preceding except that there are four prothallial nuclei instead of one; it is uncertain whether they have been derived from one or two primary prothallial cells.—FIG. 4. A gametophyte with two primary prothallial cells (p_1, p_2) which have not divided, though the pollen is presumably ready for shedding.—FIG. 5. A gametophyte with a large stalk cell placed transversely to the axis of the body cell.—FIG. 6. Same as preceding except that there are four derivatives of the primary prothallial cell.—FIG. 7. A gametophyte in which the origin of the free nuclei is not easily traced; see text for discussion.

cells contains four cells. The second one almost always divides once and usually twice. Thus there may be one, three, four, six, or eight prothallial cells; six is much the commonest number, and one or eight the rarest. In case only one prothallial cell is cut off, its nucleus

remains large and the cell itself is often much larger than when two are cut off (*text fig. 1*). Sometimes the three resulting nuclei then lie in a row across the spore (*text fig. 1*) and differ but little from one another in appearance. The walls separating them then run nearly straight across, and it is only by courtesy that the tube nucleus can be said to be free in the spore cytoplasm. To all intents and purposes it is as much walled-off into a cell of its own as the prothallial nucleus, for both eventually are freed in a common cytoplasm (*text fig. 2*). Further, it is to be noted that in this case the prothallial cell does not have a fixed position with respect to the wings, as in the ordinary course of events.

After the tube nucleus has been cut off, the primary spermatogenous cell divides transversely, giving rise to two cells (*fig. 25*). Usually these cells differ markedly in size, the smaller lying to one side and the larger lying nearly in the center of the pollen grain (*fig. 26*). In this case one may safely use the usual terms for them and speak of the larger as the body cell and the smaller as the stalk cell. It not infrequently happens that the two derivatives of the generative cell are about equal in size (*figs. 24, 27; text figs. 5, 6*), though even then one is centrally placed and the other laterally. Before the division of the generative cell, the second primary prothallial cell has usually divided at least once. In this case the generative cell often sinks down among its derivatives (*figs. 23, 27*). The stalk cell is sometimes placed transversely to the body cell (*text figs. 5, 6*). Whether these large stalk cells will produce male cells or not can only be conjectured, though the fact that some of them seem to retain their cytoplasmic envelope, almost as distinct from the cytoplasm as the body cell itself, up to a certain early stage, would lead one to suppose that in some cases they might do so. *Text fig. 7* may show three derivatives of the generative cell and two prothallial cells in addition to the tube nucleus. One of the three derivatives of the generative cell has no cytoplasmic sheath and is probably a stalk nucleus; the two lying close together are ensheathed, though I have been unable to ascertain definitely whether each has a distinct sheath or whether both lie in a common sheath, or whether perhaps one of them is not merely beneath the cytoplasmic sheath of the other. If either the first or second supposition is true, there still remains the question

whether the two cells are body cells or male cells. If the third supposition is true then there are four prothallial nuclei, a generative cell, and the tube nucleus.

In a number of preparations there seemed to be a cell on the side of the body cell opposite the stalk cell (*fig. 27*), but in no case could I satisfy myself of the presence of a nucleus; though in view of the figures published by JEFFREY and CHRYSLER it was diligently looked for. In *fig. 27* is shown a mass at one end of this "cell," which may stand for a degenerating nucleus, although I think not. It is very indistinct and may very well be a mere aggregation of slightly denser cytoplasm. By constructing a model of the cell complex, where the body cell has sunk down between the cells of the second tier of the prothallus, and then cutting it so that the section will pass about centrally through stalk and body cell and include most of one-half of them and take a slice off the prothallial cells lying beyond them, it is possible to get preparations which show cell walls in the position shown in the figure. Hence it is not certain that in this species there are two lateral derivatives of the generative cell, as seems to be the case in the species mentioned by JEFFREY and CHRYSLER (**4**).

The dividing nuclei of the species of Podocarpus here investigated show uniformly twelve chromosomes in cases where they could be certainly counted (*figs. 14, 27, 32*), and twenty-four were counted several times, with less certainty, in the sporogenous tissue of *P.* sp. In this it conforms to the count for all other gymnosperms so far as known except Taxus, with eight and sixteen (STRASBURGER **12**), and Sequoia, with sixteen and thirty-two (LAWSON **7**).

It is not possible to speak with certainty concerning the stage at which the pollen is shed without knowing over how long a time shedding continues, and without actually having gathered pollen as it is shed naturally. But from cones that were apparently ready to shed their pollen, stages were obtained in *P. nivalis* running from the microspore to gametophytes with two prothallial cells, generative cell, and tube nucleus. It often happens that both extremes may be found in the same sporangium. In *P.* sp. no cones were apparently old enough to shed their pollen, though the oldest had essentially the same structure as those of the species just mentioned. In the cones of *P. totarra Hallii* the range appears to be still wider, for the oldest

stages shown in the plates (*figs. 26, 27; text fig. 7*) occurred along with the youngest. In some cases the cell walls were still intact, while in other pollen grains not more advanced, the nuclei and body cell were free in the common cytoplasm (*text figs. 1, 3, 7*).

Discussion

The facts presented in the preceding account are in the main confirmatory of those already reported by COKER and by JEFFREY and CHRYSLER for other species of Podocarpus; hence their main value is that they offer additional evidence that the phenomena described by these authors are not limited to the species studied by them. These authors and others as well have called attention to the resemblances to the Abietineae in the winged pollen, thick sporangium wall, and general similarity of the staminate strobilus.

The main interest centers about the development of the male gametophyte. COKER (**2**) reported two primary prothallial cells in *P. coriacea*, which divide amitotically. JEFFREY and CHRYSLER (**5**) have recorded as many as eight in the species studied by them. Miss YOUNG (**14**) has also recorded as many as four in species of Dacrydium. THOMPSON (**13**) has found 30 to 40 prothallial cells in Agathis; and LOPRIORE (**8**) has reported a cell complex in Araucaria that reaches about 15 in number before the walls break down, when the freed nuclei may then further divide until as many as 44 may be found in the pollen tube. He interprets these as spermatogenous cells and cites the case of Cupressus as analogous. CHAMBERLAIN (**2**) has already called attention to the fact that the drawings seem to indicate that these nuclei are really prothallial and that there is only one body cell. In this view Miss YOUNG (**14**) has concurred, as it would seem most students of gymnosperms must. RENAULT (**10, 11**) and OLIVER (**9**) have described the multicellular pollen grains of the Cordaitales and Stephanospermum, where as many as 20 cells are found in the male gametophyte; whether they are prothallial or spermatogenous is uncertain.

CALDWELL (**1**) has reported as many as nine or ten body cells in Microcycas, along with a single stalk and single prothallial cell. CHAMBERLAIN in a forthcoming paper reports that Ceratozamia occasionally has four sperms. JUEL (**6**) found a variable number

of sperm cells (it is not clear whether he means body cells or male cells) in *Cupressus goweniana*.

It thus seems to be an established fact that more than two prothallial cells and more than two sperms occur in widely unrelated gymnosperms. Though we may all be willing, perhaps, to accept this statement of facts, there is no such unanimity of opinion as to the interpretation of them. It is obvious that one may adopt either of two views: (1) One or two prothallial cells were found in the primitive gymnosperms and in some cases these have divided to form a complex. This would be JEFFREY'S (**5**) "coenogenetic proliferation." In support of this view he has urged that a multiplication of prothallial cells is correlated with "protosiphonogamic" fertilization in the Araucarineae, "since the length of the pollen tube, in the absence of any special conductive tissue, such as is found in angiosperms, calls for a greater development of prothallial tissue." This line of reasoning could hardly be extended to Podocarpus or to any other case now known. If one could find some physical cause applicable to all cases, he might believe that this multiplication of cells is a thing of recent origin in each case. Furthermore, the idea that a multiplication of cells would be forthcoming just when needed is more teleological than accords with modern physiological teaching. If we do not accept this explanation, as I think we cannot, we may turn to the other possibility. (2) Primitive gymnosperms had a multicellular prothallial tissue, and not unlikely a spermatogenous complex of several cells. The evidence for this view is of two sorts, historical and theoretical. Historically we know that the pollen grains of certain Cordaitales (**10**) and Cycadofilicales (**9, 10, 11**) had a considerable tissue of some sort. Whether it was spermatogenous or prothallial is not very material, since this view assumes that both sorts existed somewhere in the ancestry of living gymnosperms and that by a gradual reduction each sort has been reduced to one or two cells. It is of interest to note that all the heterosporous pteridophytes show the same tendency, each of the living genera having a single prothallial cell and in some cases producing as few as four sperms. This assumption makes it easy to account for the facts already related. We have but to suppose that in these cases the forms showing these peculiarities have merely retained their primitive

characters or, in cases where they occur only occasionally, that it is a reversion to ancestral conditions. JUEL (6) has already suggested this explanation for the case of Cupressus, and has even gone so far as to suggest a possible sequence in the reduction. At this point one must be on guard against supposing that types which illustrate this reduction series necessarily, or even probably, stand in the same series phylogenetically. It seems to the writer that if this fundamental principle be firmly grasped, it is entirely unnecessary to suppose that because Podocarpus and Pinus are clearly related and because Pinus is older historically, therefore the multiplication of prothallial cells in Podocarpus must be coenogenetic. One need but assume that both are derived from a common ancestral stock and that one has retained the prothallial complex and the other has lost it. Of course if one believes that Podocarpineae have come directly out of living Abietineae, this explanation would not hold. While it is true that the latter are known as far back as the Carboniferous (4) and the former are not known to be nearly so old, it is equally undeniable that we know but little of the plant remains of those parts of the world in which their remains would be most likely to be found.

Summary

1. There are in the species of Podocarpus studied two prothallial cells which may or may not divide. There may be as many as eight prothallial cells in two tiers derived from the two primary ones.

2. Division in the prothallial tissue is mitotic and the prothallial cells do not degenerate.

3. There is a stalk cell and a body cell, sometimes differing very little from one another in appearance; whether both may produce male cells is yet uncertain.

4. The number of chromosomes is twelve and twenty-four.

5. There may be a variable number of cells or free nuclei in the pollen grain at the time it is shed.

Acknowledgments are due to Professors JOHN M. COULTER and CHARLES J. CHAMBERLAIN of this laboratory for valuable advice and criticism during the progress of this investigation.

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EXPLANATION OF PLATES VIII AND IX

The drawings were made with a Bausch and Lomb camera lucida and Zeiss apochromatic lenses and compensating oculars. The plates have been reduced one-half in reproduction.

PLATE VIII

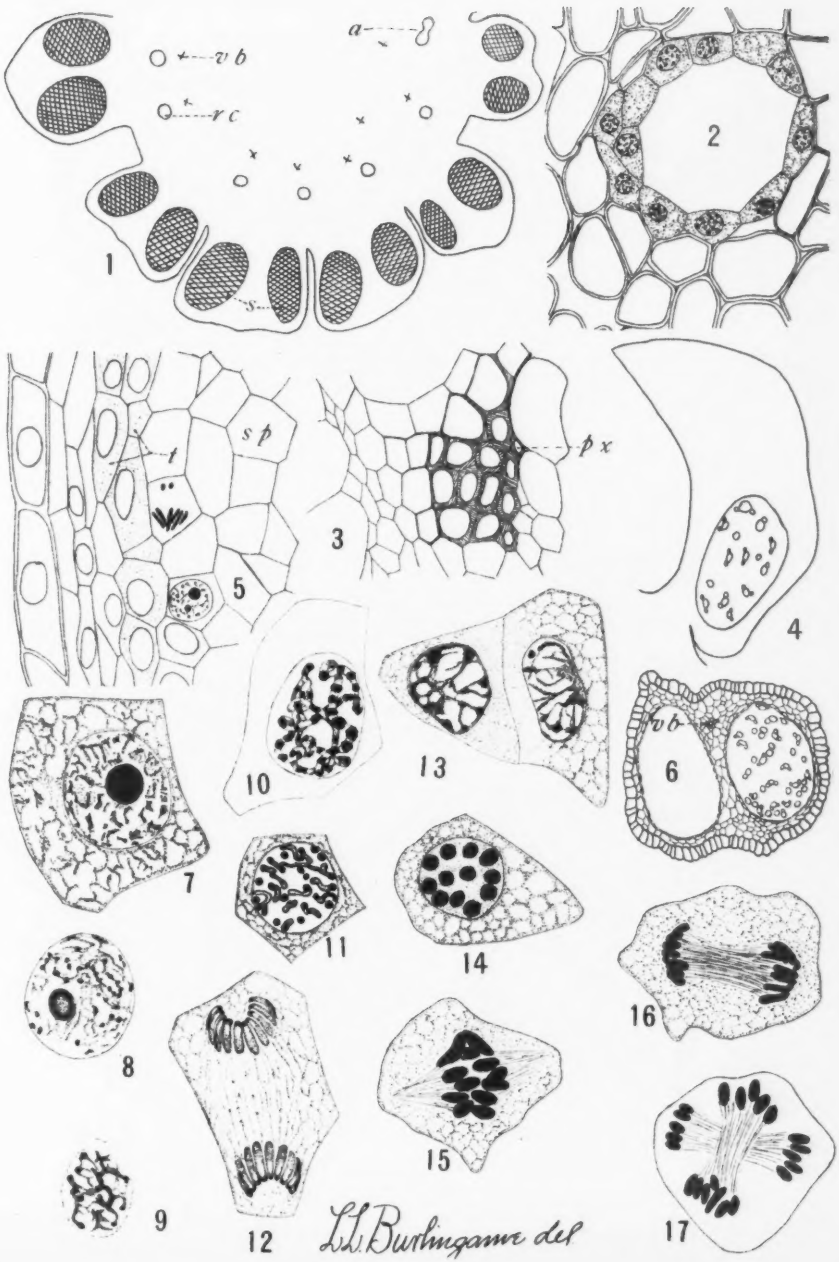
(*Figs. 1, 2, 5, 7–17* are *P.* sp.; *figs. 3, 4, 6* are *P. totarra Hallii*)

FIG. 1.—Cross-section through the middle region of a staminate cone, showing the relative position of sporophylls and sporangia (*s*), vascular bundles (*vb*), and resin canals (*rc*); the canals sometimes branch as shown at *a*.

FIG. 2.—Cross-section of resin canal.

FIG. 3.—Cross-section of vascular bundle from strobilus; *px* shows position of protoxylem.

FIG. 4.—Longitudinal section of a sporophyll, and one sporangium of a young cone about 3^{mm} in length.


a
v b
v c
1
2
s p
t
5
3
p x
4
10
13
6
v b
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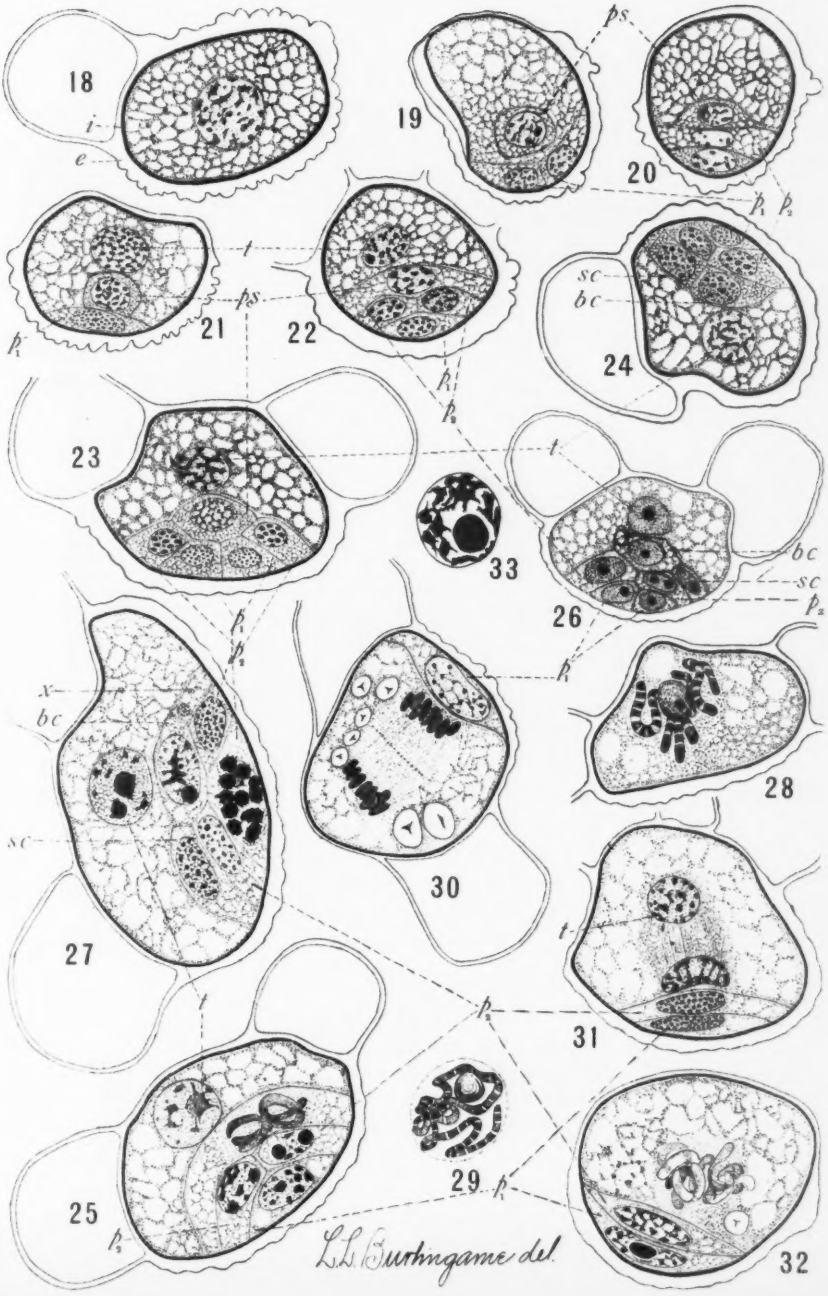


BURLINGAME on PODOCARPUS

FIG. 5.—Detail of sporangium of same age as preceding, showing relations of wall, tapetum (*t*), and sporogenous tissue (*sp*).

FIG. 6.—Cross-section through sporangia; *vb*, vascular bundle.

FIG. 7.—Cell of the sporogenous tissue about one division short of the mother cell stage.

FIG. 6.—Detail of a nucleus a little later than preceding, showing the nuclear materials beginning to arrange themselves into threads with here and there deeply staining chromatin knots.

FIG. 9.—Surface view of a still older nucleus showing the strands more distinct and larger.

FIG. 10.—The definitely organized spirem with unusually distinct chromatic knots; the spirem is usually more homogeneous and stains more evenly.

FIG. 11.—A very thin section of a nucleus whose spirem is beginning to segment into chromosomes; compared with *fig. 7*, it shows the variation in size and shape of the sporogenous cells.

FIG. 12.—Longitudinal section of a cell in telophase showing about half of each group of chromosomes; the spindle is curved with the concavity toward the observer.

FIG. 13.—Showing the reconstruction of the daughter nuclei.

FIG. 14.—Mother cell showing the emergence of the chromosomes from the granular mass out of which the spindle arises; the granular materials are actually much denser than shown in this figure and nearly conceal the chromosomes.

FIG. 15.—Longitudinal section of the heterotypic spindle.

FIG. 16.—Late anaphase of the heterotypic mitosis.

FIG. 17.—The homotypic mitosis.

PLATE IX

(*Figs. 18–27, 33* are *P. totarra Hallii; figs. 28–32* are *P. nivalis*)

FIG. 18.—Section of microspore slightly oblique to the wings; *i*, intine; *e*, exine.

FIG. 19.—Male gametophyte showing first prothallial cell (p_1) divided, no second prothallial (p_2) cell, primary spermatogenous cell (*ps*), and in next section the tube nucleus (*t*).

FIG. 20.—Same stage as preceding except that there are here two primary prothallial cells (p_2), the second of which has divided (only one, p_2, of the segments shown).

FIG. 21.—Same as *fig. 19* except that the single primary prothallial cell (p_1) has not divided; *t*, tube nucleus.

FIG. 22.—Male gametophyte showing first prothallial (p_1) undivided, second $(p)_2$ divided once, primary spermatogenous cell (*ps*), and tube nucleus (*t*).

FIG. 23.—Same as preceding except that first prothallial cell (p) has also divided and the primary spermatogenous cell (*ps*) has sunk down between the derivatives of the second prothallial cell (p_2); adjacent sections show that there are here four derivatives of the second prothallial cell.

FIG. 24.—Showing the stalk (*sc*) and body (*bc*) cells as well as prothallial cells (p_1, p_2) and tube nucleus (*t*).

FIG. 25.—The mitosis giving rise to stalk and body cells from the primary spermatogenous cell.

FIG. 26.—A usual type of mature gametophyte, having four derivatives of the first primary prothallial cell (p_1), four of the second (p_2), stalk (*sc*), and body (*bc*) cells, and tube nucleus (*t*); nine cells also usual (two p_1, four p_2, stalk, body, tube).

FIG. 27.—Showing the prophase of the division of the first prothallial cell (p_1) and what appears to be a "cell" (*x*) on the side of the body cell opposite to the stalk cell (*sc*); no nucleus could be discovered in this "cell;" see text for further details.

FIG. 28.—Division of the microspore nucleus in *P. nivalis*; a peculiar segmented appearance of the spirem, which has about completed segmentation into chromosomes, is shown.

FIG. 29.—A similar but younger spirem which has not yet begun to segment as the preceding one has done.

FIG. 30.—Anaphase of the division resulting in second prothallial cell (p_2) and antheridium initial.

FIG. 31.—Late telophase of division of antheridium initial into primary spermatogenous cell and tube nucleus; this is the usual shedding stage of this species, with prothallials undivided.

FIG. 32.—Metaphase of division of antheridium initial showing the twelve gametophytic chromosomes; see also *fig. 27*.

FIG. 33.—Details of nucleus of primary spermatogenous cell of *P. totarra Hallii*.

SISYRINCHIUM: ANATOMICAL STUDIES OF NORTH AMERICAN SPECIES

THEO. HOLM

(WITH PLATES X, XI, XIA)

Before the genus Sisyrinchium becomes entirely lost in segregates and in new species, which in late years have accumulated very rapidly, it might be appropriate to present some notes to demonstrate the characteristics of the genus as represented within our own boundaries.

Three sections are recognized by BENTHAM and HOOKER: BERMUDIANA Adans., ECHTHRONEMA Herb. (HYDASTYLUS Salisb.), and ERIPHILEMA Herb., based on floral structure, and especially whether the filaments are connate for their entire length or only partly so. In his work on the *Botany of California* WATSON reached the same conclusion, and considered these floral characters as being merely of sectional importance. But in late years some American writers have raised these sections to generic rank, though with the preference of Sisyrinchium for Bermudiana, and Hydastylus for Echthronema, and without having invented any other distinctive characters than the floral, already described by BENTHAM and HOOKER, and by WATSON. In fact the habit of these plants is very much alike; and strikingly so when compared with Iris, in which genus the floral structure exhibits no small modification, and besides, the habit is extremely variable; nevertheless Iris has not yet suffered the same dissolution as Sisyrinchium.

The internal structure of the Irideae has already been studied by some authors, but not very extensively, since only the leaves have been examined. ROSS,[1] and CHODAT and BALICKA-IWANOWSKA[2] have published some very interesting anatomical papers dealing with the foliar structure of a large number of genera, but very little has been written in regard to our genus Sisyrinchium. The following notes may thus be considered as supplemental to these papers, but I have included the structure of the stem and root for the sake of

[1] Anatomia comparata delle foglie delle Iridee. Malpighia 6: 1892; and 7: 1893.

[2] La feuille des Iridées. Jour. de Botanique. 1892.

making the anatomical diagnosis a little more complete. Moreover, I believe that the internal characters observed in representatives of the three sections mentioned above will strengthen the former view of considering Sisyrinchium as one genus, and a very natural one.

The material examined consists of *S. anceps* Cavan., which is very frequent in sandy soil among rocks on the Potomac shore (D. C.); *S. angustifolium* Mill., from sandy hills near Brookland (D. C.); *S. montanum* Greene, from gravelly soil along creeks on Long's Peak (Colorado), at an elevation of about 9000 feet; *S. xerophyllum* Greene, which I found quite abundant in low pine barrens near Eustis (Florida); *S. grandiflorum* Dougl., from dry hillsides in Oregon; and *S. californicum* Ait. f. from swamps at Bodega (Cal.). The last two species were kindly sent to me by Miss ALICE EASTWOOD and Mr. THOMAS HOWELL. To these may be added the tuberous-rooted *S. alatum* Hook. from lava fields above Cuernavaca, Morelos, Mexico, at an elevation of 8500 feet, collected by Mr. C. G. PRINGLE.

As stated above, the general habit of these species is very much the same, especially the inflorescence. This represents in our species of Sisyrinchium a rhipidium,[3] and this type of monochasium is frequent among the Irideae. Whether the inflorescence is single and terminal or accompanied by a lateral, the flowers are always surrounded by green, leaflike bracts (*L*, L^1, and L^2, *fig. 2*); a membranaceous foreleaf occurs at the base of the lateral inflorescence (*P*, *fig. 2*) and at the base of the axillary flowers (P^1–P^3, *fig. 6*). The successive development of the flowers may be seen in the diagram (*fig. 6*), where five flowers (*1–5*) are situated in the same plane, and where *1* is the terminal, the earliest developed. The various stages of these flowers are shown in *figs. 3–5*, but the two bracts (L^1, L^2) are only indicated. The stamens, especially the anthers (*St*, *figs. 4, 5*), are much farther advanced than the outer (P^1) and inner perianth leaves (P^2). I have found no deviation from this type of inflorescence in any of the three sections, but the relative length of the green bracts (L–L^2, *fig. 2*) varies very much, and is considered as a specific character, with no good reason, however. Bracts and

[3] Compare EICHER, Blüthendiagramme 39. *fig. 20A*.

foreleaves, as we know, are very frequently subject to variation, sometimes in accordance with the surroundings

The rhizome is usually very short and cespitose, but in some species, *S. californicum* for instance, the rhizome is horizontally creeping, with the internodes quite distinct. The leaves are two-ranked, equitant, and the aerial stems strongly compressed, except in *S. grandiflorum*, in which leaves and stems are almost cylindric. It would be interesting to know whether the species of these three sections germinate in the same way, but so far this point does not seem to have been studied. For this reason I have followed *S. angustifolium*, which is so very frequent in the vicinity of Brookland, from seedling to mature plant, and the structure of the seedling is as follows. The cotyledon (*Cot, fig. 1*) is epigeic and consists of a sheathing base and a long filiform blade, the apex of which remains inclosed within the seed for some time, as shown in the accompanying drawing. Three leaves (L^1–L^3) are developed from the plumule during the first season, and the structure of these leaves agrees with that of the later ones, being ensiform and green. The primary root (*R*) grows and remains active during the first season, and becomes ramified; secondary roots (*r*) develop also, and these proceed from the base of the cotyledon. It requires several years for the plant to reach the flowering stage, and until then the small rhizome remains as a monopodium similar to Iris. This method of germination represents KLEBS' type 5,[4] and corresponds, to some extent, with that most frequent among the dicotyledons where the seed leaves are epigeic. It is characteristic of several Liliaceae (Allium, Bowiea, Asphodelus, etc.) and of Agave, but not of Iris; in *I. Pseudacorus*, for instance, the apex of the cotyledon remains inclosed within the seed, and does not become free as in Sisyrinchium. So far as the external structure of the various members of the three sections mentioned above is concerned, there seems no plausible reason for dividing the genus, and we shall see that the internal structure also does not warrant any such segregation. Whatever distinctive characters have been observed are merely sectional, and it is hardly necessary to call attention to the fact that much more pronounced deviations in struc-

[4] Beiträge zur Morphologie und Biologie der Keimung. Untersuch. Bot. Inst. Tübingen 1:572. 1881–1885.

ture are known in many other genera, which so far have been left intact.

Anatomically our genus is not a very interesting one, but the structure has never been studied, and I thought that the following discussion of the vegetative organs might be of some interest from a comparative point of view. Some knowledge of the structure, especially since the material came from widely separated stations, may be of interest to students of plant societies, for it seems to me that many of the conclusions reached in the name of ecology are too superficial, so long as the plants themselves have not been studied thoroughly.

ROOTS

In our native species of Sisyrinchium the secondary roots are slender; they are soft and of a whitish color in *S. californicum*, but quite strong, and yellowish brown in the other species. In *S. xerophyllum* and *S. grandiflorum* the roots are almost villous from the dense covering of root hairs, while in the other species the roots are much less hairy. The epidermis is thin-walled, and inside this is an exodermis of a single stratum, whose cells are mostly pentagonal in cross-sections, and thin-walled in all the species except *S. xerophyllum*, in which the exodermis is very distinctly thickened. The cortical parenchyma contains no stereids, and is composed of a compact, but thin-walled tissue; in *S. montanum* the cortex collapses tangentially, but remains solid in the other species, and contains deposits of starch except in *S. californicum*. The endodermis shows a very pronounced thickening of the inner cell walls, thus representing a U-endodermis, but is thin-walled in *S. californicum*.

The pericambium consists generally of a single layer of thin-walled cells (*P*, *fig. 8*), and is continuous; however, the following exceptions were noticed. In *S. montanum* (*fig. 9*) there is frequently a second stratum of pericambium to be observed outside some of the hadrome rays and this same condition occurs also in *S. xerophyllum* (*fig. 10*). Sometimes the pericambium becomes thick-walled, as may be seen from *fig. 11*, which is of *S. xerophyllum*, and in which the cells outside the leptome are quite thickened in contrast to those outside the hadrome. In these same figures of *S. xerophyllum* (*figs. 10, 11*) we notice also that the pericambium is not continuous, but

interrupted by the protohadrome vessels. This interruption of the pericambium was observed only in this species (*S. xerophyllum*), in all the roots examined, but not in any of the roots of the other species.

The hadrome is very conspicuous in all the roots, and there may be as many as twelve rays with two or three protohadrome vessels in each ray; the leptome, on the other hand, consists of very narrow strands, in which the protoleptome cell is readily visible (*PL, figs. 8–11*). Although the rays of the hadrome extend to the center of the root, the conjunctive tissue, nevertheless, is very conspicuous, and is mostly thin-walled except in *S. xerophyllum* and in some of the roots of *S. anceps*.

This structure seems to be characteristic of the slender secondary roots of mature specimens of this genus. In the corresponding roots of the seedling of *S. angustifolium* no exodermis is developed and the cortical parenchyma is very thin-walled; the endodermis, on the other hand, is very thick-walled, representing a typical U-endodermis. In these young roots the pericambium is continuous, and the stele contains only two hadromatic rays alternating with two small groups of leptome. In regard to the structure of the stele, the pericambium, and the mestome, the thin lateral roots of mature specimens agree with that observed in the secondary ones of the seedling; but the endodermis is different, being thin-walled and showing the Casparyan spots very plainly (*End, fig. 16*).

The structure of the roots of these species is thus very uniform, and the only distinction seems to depend upon the structure of the pericambium, which sometimes consists of more than one stratum, and which may be interrupted by the protohadrome, as observed in *S. xerophyllum* from subtropical Florida.

In the tuberous roots of the Central American *S. alatum* Hook. the cortical parenchyma represents a very large parenchyma filled with starch, and with the cell walls thickened; but otherwise the structure of epidermis, exodermis, endodermis, and pericambium, which is continuous, is identical with that of the species described above. The number of hadromatic rays averages about twelve, and the leptome occurs as exceedingly narrow strands in these tuberous roots.

RHIZOME

S. californicum is the only species examined that possesses a horizontally creeping rhizome with the internodes distinct. The structure is rather weak when compared with that of the rhizomes of the monocotyledons in general, since no stereome is developed. The epidermis is thin-walled and covers a cortex of broad, compact parenchyma; the endodermis is thin-walled, and the cells somewhat irregular in shape. Inside the endodermis is a large, thin-walled pith, in which the numerous mestome strands are located, but without being arranged in bands; most of these mestome bundles were observed to be leptocentric, the leptome being more or less completely surrounded by the hadrome.

STEM ABOVE GROUND

With exception of *S. grandiflorum* the stem is ancipital, simple, or sometimes branched above; the wings vary somewhat in breadth, but the central portion of the stem is always cylindric, and of a very firm structure, due to the presence of a solid sheath of thick-walled stereome. The cuticle is frequently thick and wrinkled, and cell walls of the epidermis are prominently thickened, especially the outer, and very often extended into short papillae, but not in *S. californicum*. In the body of the stem as well as in the wings the cortex is composed of palisades and represents a very compact tissue, but in *S. californicum* the cortical parenchyma is more open and the cells very short, almost roundish in transverse sections. There is no hypodermal collenchyma or stereome in any of these species, and the stereome constitutes, as stated above, a closed sheath of several strata located on the inner face of the cortex in the cylindric portion of the stem; in the wings the stereome accompanies the larger mestome strands as small groups on the leptome side. The mestome bundles are arranged in two concentric bands in the cylindric part of the stem, but in a single plane in the wings. They are collateral, and those of the peripheral band are very small, and contain mostly leptome; they are located in the cortex, but their hadrome touches the stereomatic sheath. Those of the inner band are much larger and are located directly inside the stereome cylinder, with their hadrome bordering on the pith. The innermost part of the cylinder

is occupied by a pith, which is frequently somewhat thick-walled and solid. In the wings the mestome strands are few, and they show mostly the same position of the hadrome, which is turned toward the center of the stem; it is only the marginal mestome strands that differ in this respect, the leptome and hadrome being vertical on the surface of the wings, instead of parallel.

In *S. grandiflorum* the structure of the stem is somewhat different; the outline is less compressed and there are many deep furrows. These furrows contain cortical parenchyma, and the peripheral band of small mestome bundles is located in the ridges, thus imbedded in the cortex and removed from the stereomatic sheath. The inner band of mestome bundles, on the other hand, is located inside the stereome, as in the other species described above.

The structure of the stem is thus very uniform in these species of Sisyrinchium, and it seems characteristic of the genus that no hypodermal mechanical tissue is developed; that there is a strong sheath of stereome separating two concentric bands of mestome bundles, of which the peripheral are of the same size and much smaller than the inner ones; furthermore, the winged stem of most of these species constitutes also a very conspicuous feature.

LEAVES

The foreleaves are membranaceous, almost destitute of chlorophyll, and strongly compressed from side to side; they are frequently scabrous from thick-walled papillae (*fig. 12*), but only on the dorsal face. The cuticle is smooth, and the epidermis is moderately thick-walled on the dorsal face, but thin-walled on the ventral. There is only chlorenchyma in the middle portion of these leaves, and it consists of roundish cells throughout, with very little chlorophyll; the broad and very thin margins of the foreleaves consist only of epidermis, that of the dorsal face. The stereome is very poorly represented; it occurs as two very small, hypodermal strands, one on each side of the ventral sinus above the midrib, and the veins have a few layers of this tissue on the hadrome side. There are several mestome strands in these leaves (3 to 8) and the midrib is usually a little thicker than the others; the mestome strands are collateral, and surrounded by parenchyma sheaths. While the leptome is well developed in

all the veins, the hadrome is frequently absent, except in the midrib.

The green leaves are sheathing, and provided with a long, strongly compressed blade, the structure of which is very markedly isolateral. By examining the anatomical structure of the blade one gets the impression that the peculiar position of the leptome and hadrome in the veins is due to a concrescence of the two halves of the leaf-blade, resulting in a more or less complete suppression of the morphologically ventral face of the blade.

However, the development of the ensiform leaf of Irideae, as described by GOEBEL,[5] teaches us something very different. According to this author the singular shape and structure of the blade is caused not by a concrescence of the wo halves of the blade, but by the peculiar growth of the leaf-primordium. The young leaf becomes laterally compressed at a very early stage of its development, and it so happens that its dorsal face shows this flattened growth much more than the ventral. The real growing-point soon ceases to be active, while a new point takes its place and this is located on the extreme back of the primordium, and somewhat lower than the original. The leaf thus shows two apices, and the secondary of these grows out into a long bladelike organ, which consequently has no ventral face, and in which the arrangement and structure of the veins must follow other laws than in a normally developed leaf-blade. The real apex of the leaf is thus to be found at the upper part of the open sheath, while the bladelike organ represents merely a secondary growing-point, which has surpassed the primary one.

In the species of the section BERMUDIANA the leaf-sheath is usually much shorter than the ensiform blade; in the section ECHTHRONEMA, on the other hand, the sheath may be traced to very near the apex of the blade; and finally, in the section ERIPHILEMA the blade is less compressed and often much shorter than the open, sheathing portion of the leaf. It is interesting to see that although the leaves are ensiform in all these species, the internal structure nevertheless exhibits two types so far as concerns the disposition of the mestome strands, which are in a single plane, as in BERMUDIANA, or in two parallel planes, as in the two other sections. But otherwise the

[5] In SCHENK'S Handbuch der Botanik 219. Breslau. 1884.

structure is rather uniform, and the few distinctions which I have observed seem to be of merely specific importance.

Beginning with section BERMUDIANA, the leaf-blade is smooth in *S. anceps*, but furrowed longitudinally in several of the other species, as *S. angustifolium*, *S. bellum*, etc. The surface varies from perfectly glabrous to very prominently scabrous, and it deserves notice that the leaves may exhibit both structures when examined at different places. The apex may be very scabrous, for instance, in contrast with the lower parts of the blade, or sometimes *vice-versa;* or some of the earliest developed leaves may be more glabrous than the succeeding. I mention this since the characters "glabrous" and "scabrous" figure so very conspicuously in the recently published diagnoses of "new species of Sisyrinchium."

The cuticle is thick and distinctly wrinkled. The epidermis is frequently thick-walled, especially the outer walls (*figs. 13, 15, 20, 21*), and, as stated above, the extension of epidermis into papillae of various forms, pointed or obtuse, is common to several members of the genus (*fig. 21*).

The stomata are arranged in longitudinal lines on both sides of the blade, and they are sunk (*fig. 13*). It is interesting to notice that in the subtropical *S. xerophyllum* (from Eustis, Florida) the thickness of epidermis reaches its maximum (*fig. 15*); the cell walls are extremely thick and porous, but no papillae were observed in this species.

In regard to the chlorenchyma, we have in this section a more or less typical and compact palisade tissue of several layers, the innermost of which forms circular bands around the mestome bundles, with the cells radiating toward the center; while in the peripheral (hypodermal) strata the palisade cells are vertical to the epidermis. In some species, for instance *S. anceps* and *S. xerophyllum*, the chlorenchyma breaks down and forms lacunes between the veins, especially in the latter species. The mechanical tissue in the genus is only represented by stereome, which accompanies the mestome bundles as a small cover on the leptome and hadrome side; but it is not very thick-walled except in *S. xerophyllum*, and it does not occur as isolated, hypodermal groups in any part of the leaf. It occupies the somewhat unusual position of being located inside the thin-walled, chlorophyll-bearing parenchyma sheath, which

surrounds each of the mestome strands. However, this parenchyma sheath differed from similar sheaths in other monocotyledons by showing no resistance when treated with concentrated sulfuric acid.

The arrangement of the mestome strands is very peculiar, and especially the disposition of leptome and hadrome. From the fact that the blade is not a blade in the proper sense of the word, but an outgrowth of the dorsal face, hence with no ventral face developed, the course of the veins is different from that of leaves in general. If we examine a cross-section of the blade we notice at once that all the mestome strands occupy a single plane, extending from the one margin of the blade to the other; also that the mestome strands show a different position of leptome and hadrome in relation to the periphery of the section. Those near the center of the blade are the largest, and in these the leptome turns alternately with the hadrome to the right or left of the longitudinal axis of the leaf; in this way, by examining the two sides of the leaf, we find in one mestome strand the leptome turned toward the right, in the next toward the left, etc.; and the same alternating position is of course also occupied by the hadrome. But the small strands, which are located in the thin margins of the blade, show almost constantly the leptome turning toward the edge of the leaf, and the hadrome, on the contrary, toward the center. In other words, the structure of the blade looks as if the two halves had grown together, but we know from the development of the leaf-primordium that no such concrescence has taken place. The mestome bundles are collateral and show the ordinary structure; anastomoses are not infrequent, and they form very acute angles with the larger veins, as in Eriocaulon for instance. The leaf structure is thus very uniform in this section, and the most conspicuous variation seems to depend upon the thickness of the epidermis, and upon whether the chlorenchyma forms typical palisades (*fig. 20*), or consists of short, almost roundish cells, as in *S. anceps* (*fig. 21*).

In *S. grandiflorum* (section ERIPHILEMA) the leaf structure differs very conspicuously from that of the former section, and resembles much more the structure of a stem than of a foliar organ. In this species the leaf is less compressed; the mestome strands constitute a narrow elliptical band, instead of being located in a single plane;

also the central portion of the leaf contains a thin-walled, colorless tissue very much resembling the pith of a stem, and is frequently hollow. Otherwise the structure of the tissues is identical with that of several species of the former section (BERMUDIANA). The cuticle is thick, but smooth; the epidermis is papillose, moderately thick-walled, and the chlorenchyma is composed of palisades, which are vertical to the epidermis but very open from wide intercellular spaces. The stereome is rather thin-walled, and shows the same distribution as in BERMUDIANA. The leaf is very distinctly furrowed longitudinally, and the mestome strands, which are of two sizes, are arranged alternately so that the smaller are in the furrows and the larger in the ridges. All the mestome strands turn the leptome toward the periphery of the blade, while the hadrome borders on the thin-walled parenchyma, which fills the innermost portion of the blade, thus resembling a pith in respect to structure and position.

In the section ECHTHRONEMA the principal structure of the blade agrees better with that of ERIPHILEMA than of BERMUDIANA; because the mestome strands are here also in a very narrow elliptical band with the hadrome turned inward and bordering on a central, thin-walled parenchyma. In *S. californicum* the cuticle is thin and smooth; the epidermis is relatively thin-walled and perfectly glabrous. The chlorenchyma (*fig. 18*) shows no palisades, but is composed of a homogeneous tissue of oblong to roundish cells (in cross-section); however, a superficial section of the blade (*fig. 17*) shows the cells of the chlorenchyma very distinctly stretched and lobed, not parallel with the longitudinal axis of the leaf, but vertical to it. In this way the chlorenchyma shows actually the structure of a pneumatic tissue, as this is developed in the dorsal portion of leaves; the central part of the chlorenchyma in this species is also a colorless, thin-walled parenchyma. No stereome was observed, and the mestome bundles are in a narrow elliptical band; they are collateral and surrounded by thin-walled parenchyma sheaths.

This is in brief the anatomical structure of these species of Sisyrinchium, and bearing in mind that representatives of each of the three sections have been examined, it appears to me that the genus is a very natural one, and that it ought not to be divided. In regard to the morphological structure of the shoot, I have not been able to

detect any character that seems peculiar to these sections; on the contrary, the rhizome, the leaves, and the inflorescence are very uniformly developed, and the only distinction depends upon the floral structures which were mentioned in the introduction. If these floral structures were accompanied by differences in the development of the shoot, and also by peculiarities in the internal structure, there might have been some reason for dividing the genus. There are several very peculiar looking species in Mexico, and these ought to be examined anatomically from fresh material. Until then it seems most advisable to leave the genus intact.

Finally, I wish to add a few words in reference to the structure of the chlorenchyma in other genera of the Irideae. We have seen the chlorenchyma in the sections BERMUDIANA (*fig. 19*) and ERIPHILEMA is more or less differentiated as palisades, while in ECHTHRONEMA this tissue is composed of oblong cells parallel with epidermis; moreover, that the cells in ECHTHRONEMA are lobed, resembling those of a pneumatic tissue. These structures occur also in other genera, and for the sake of making a comparison I examined some of them. In Iris, for instance, we find in *I. cristata* Ait. (*fig. 22*) a structure of the chlorenchyma which corresponds well with that of *Sisyrinchium californicum*, but the cells of which are branched rather than lobed, thus representing a typical pneumatic tissue. In *Iris verna* L. and *I. fulva* Ker., on the other hand, there are typical palisades, which, however, show the same direction, being parallel with the epidermis and vertical to the longitudinal axis of the blade; in *I. xiphioides* Ehrh. the palisades are vertical to the epidermis. A structure that corresponds with that of *Iris verna* is furthermore characteristic of *Tapeinia magellanica* Juss., *Freesia refracta* Klatt., and *Tritonia* sp. In *Belamcanda chinensis* Adans. the chlorenchyma has a very irregular structure, the cells varying from oblong and entire to more or less deeply lobed. We have thus in Iris as varied a development of the chlorenchyma as was observed in Sisyrinchium.

I have also pointed out the somewhat peculiar arrangement of the mestome strands in Sisyrinchium (the single plane in BERMUDIANA, and the narrow elliptical band in ERIPHILEMA and ECHTHRONEMA). That such variation in the position of the mestome

bundles is also observable in other Irideae may be seen from the descriptions and figures in the work of Ross cited above. It is really surprising to see the polymorphic structure possessed by Iris. In *I. alata* Poir. the leaves are perfectly open, not ensiform, and the veins show exactly the same position as in a typical linear leaf of grasses, for instance; in species with ensiform leaves we notice in *I. foetidissima* L. the mestome strands forming a narrow ellipse; in *I. juncea* Desf. they are arranged in an almost circular band; in *I. reticulata* M. B. the band is quadrangular; and finally in *I. japonica* Thunb. the structure is like that of section BERMUDIANA. A very striking variation occurs in Gladiolus, where the outline of the section varies from linear to cross-shaped with a corresponding variable position of the mestome strands. These modifications in structure are very striking, and moreover they are in several cases accompanied by distinct external characters, floral or vegetative; nevertheless they are not looked upon as being of anything but specific importance, an opinion which no doubt is the most natural.

In regard to the almost untold number of recently described "species" of Sisyrinchium, I have examined the internal structure of some of these, but so far have failed to detect any character that might appear specific; and moreover it seems very evident, when the diagnoses of the majority of these are read, that they deal not with "species" but merely with "local forms."

BROOKLAND, D. C.

EXPLANATION OF PLATES X, XI, XIA

PLATE X

FIG. 1.—*S. angustifolium:* seedling, showing cotyledon (*Cot*) with seed still attached; *R*, primary root; *r*, secondary roots; L^1–L^3, the first leaves succeeding the cotyledon. Natural size.

FIG. 2.—*S. anceps:* young inflorescence; *L*–L^2, green bracts; *P*, foreleaf of axillary inflorescence. Natural size.

FIG. 3.—*S. anceps:* inflorescence, the terminal of *fig. 2*; P^1–P^2, foreleaves; L^1–L^2, green bracts. Magnified.

FIGS. 4, 5.—*S. anceps:* two youngest flowers of same inflorescence; P^1, outer, P^2, inner perianth; *st*, stamens. Magnified.

FIG. 6.—*S. anceps:* diagram of a five-flowered inflorescence; 1–5, the flowers, of which 1 is the first developed; the other letters as above.

FIG. 7.—*S. alatum:* cross-section of root; *C*, cortex; *End*, endodermis; *P*, pericambium; three rays of hadrome and two strands of leptome are figured. ×320.

PLATE XI

FIG. 8.—*S. anceps:* cross-section of root; *PH*, protohadrome; *PL*, protoleptome; the other letters as in *fig.* 7. ×496.

FIG. 9.—*S. montanum:* cross-section of root stele; letters as above. ×496.

FIG. 10.—*S. xerophyllum:* cross-section of root stele; letters as above. ×496.

FIG. 11.—*S. xerophyllum:* cross-section of root stele; *V*, vessels; the other letters as above; many of the pericambium cells are thick-walled. ×496.

FIG. 12.—*S. angustifolium:* epidermal projection from the foreleaf. ×240.

FIG. 13.—*S. angustifolium:* cross-section of the stoma from the leaf. ×496.

FIG. 14.—*S. angustifolium:* a stoma seen from above. ×496.

FIG. 15.—*S. xerophyllum:* cross-section of leaf; *C*, cuticle; *Ep*, epidermis. ×496.

PLATE XIA

FIG. 16.—*S. montanum:* cross-section of a thin lateral root; letters as above. ×496.

FIG. 17.—*S. californicum:* chlorenchyma of leaf, seen from above.

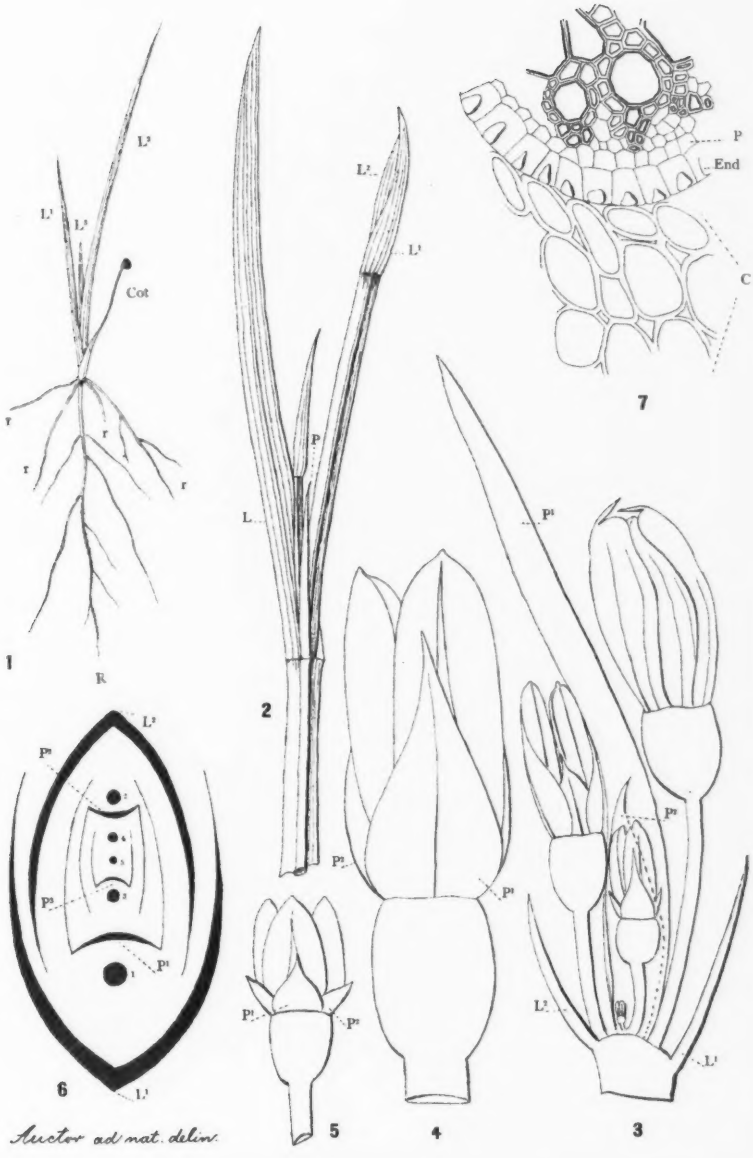
FIG. 18.—*S. californicum:* cross-section of part of leaf; *Ep*, epidermis; *PS*, parenchyma sheath of mestome strand.

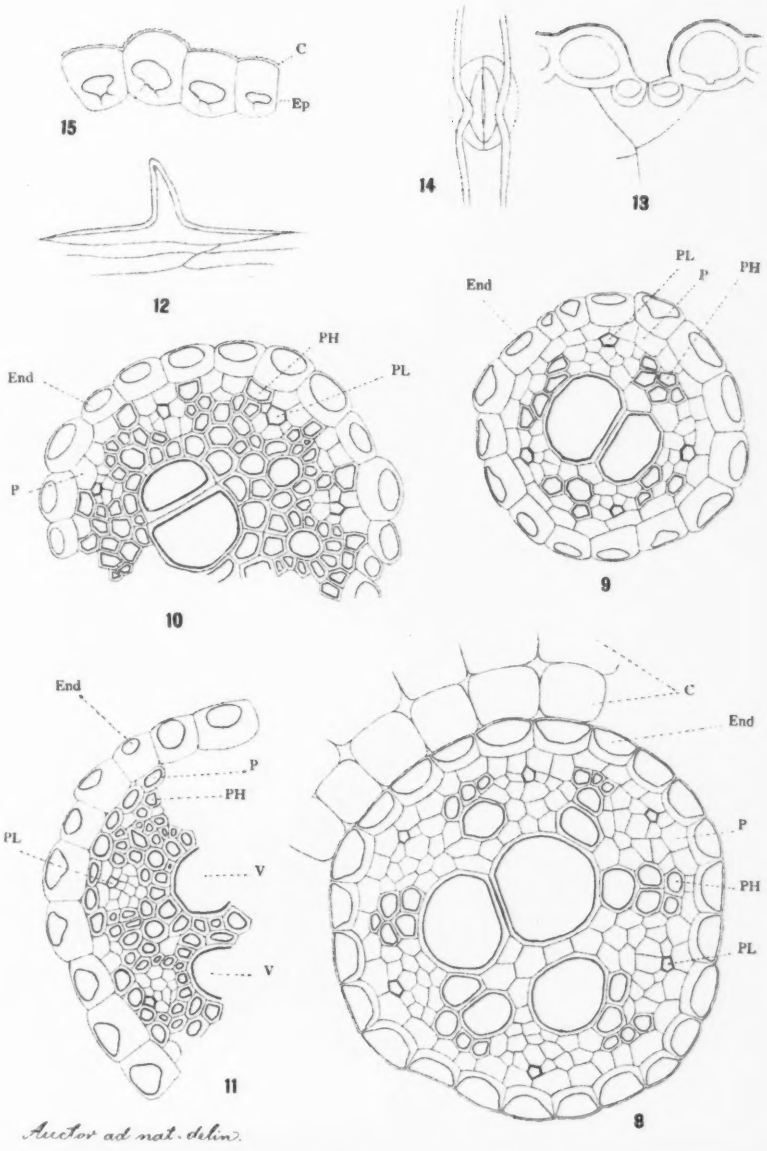
FIG. 19.—*S. montanum:* chlorenchyma of leaf, seen from above. ×240.

FIG. 20.—*S. montanum:* cross-section of part of leaf; *St*, two stomata with their air chambers (*A*); the other letters as in *fig.* 18. ×320.

FIG. 21.—*S. anceps:* cross-section of part of leaf; *Ep*, epidermis; both leptome and hadrome have a support of stereome. ×496.

FIG. 22.—*Iris cristata:* chlorenchyma of leaf, seen from above. ×240.


L¹
L²
L³
Cot
r
R
1
L²
L¹
P
L
2
P
End
C
7
P¹
P²
P¹
P³
3
L²
L¹
P¹
P³
4
P¹
P²
5
L¹
P²
P³
P¹
6
L¹
Auctor ad nat. delin.


C
Ep
15
12
14
13
PL
P
PH
End
PH
End
PL
P
10
9
End
P
PH
PL
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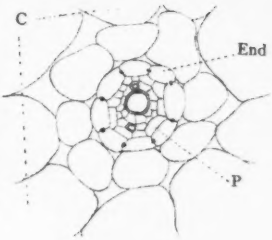
V
11
C
End
P
PH
PL
8
Auctor ad nat. delin.

19



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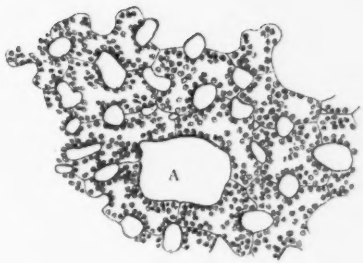

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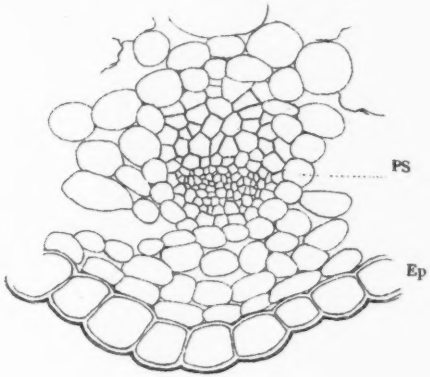

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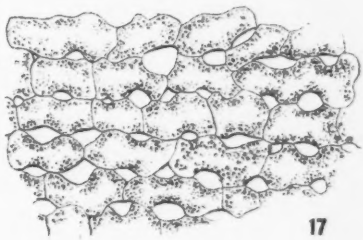

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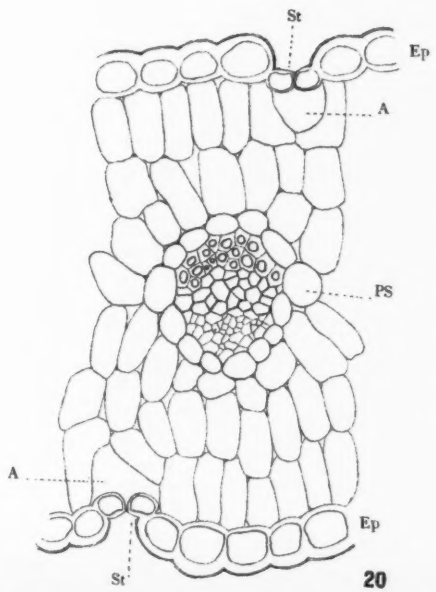

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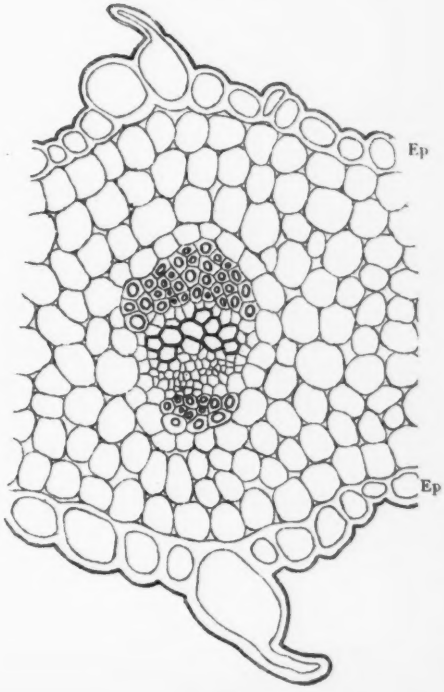

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Auctor ad nat. delin.

HOLMon SISYRINCHIUM.

A NEW RESPIRATION CALORIMETER

GEORGE J. PIERCE

It is generally known that heat is liberated, often in great quantity, whenever germination or fermentation takes place under such conditions that only a small proportion of the heat liberated is lost by radiation. For example, in the malting of barley, it is necessary to take precautions lest the temperature of the germinating grain rise too high; and California wine-makers have repeatedly told me that they are obliged to watch the temperature of the must very carefully lest the fermentation go too fast and "the yeast burn." Brewers and distillers are aware of the same fact. Botanists generally lecture about the liberation of heat in the spathes of aroids, but how many of us have seen an experiment demonstrating this phenomenon? I, for one, do not recall ever having seen one. I find that a considerable number of naturalists associate these various instances of heat liberation with rapid growth, and in some laboratory manuals of plant physiology the liberation of heat is treated in connection with growth phenomena. These same naturalists, and a good many others, think of respiration not only as involving an intake of oxygen (which is not always the case, as for example in anaerobic respiration), but also an outgo of carbon dioxid. This latter is by no means always the case, as the respiration of the sulphur, iron, and certain nitrogen bacteria shows.

The intake of oxygen and the outgo of carbon dioxid are the easiest features of the process of respiration to demonstrate in the organisms ordinarily studied in those biological laboratories in which any attention whatever is paid to live plants or animals. It is easy enough for us all to go on thinking of respiration as a sort of ventilating process, in which a poisonous waste product of the living organism is displaced or replaced by a useful gas. We do not necessarily realize how this latter gas is useful, except as it takes the place of another which is poisonous. Whether carbon dioxid is itself poisonous is, I suppose, open to dispute. At any rate, it is ordinarily of no use to the organism.

It can be used only as a food material, only by chlorophyll-containing cells, and by these only in the light.

The other features of the process of respiration are hard to demonstrate, hard even to study. The chemistry of respiration is scarcely less difficult than the chemistry of photosynthesis.[1] Whatever the reactions may be in the chain which connects the free oxygen of the air with the oxygen combined with carbon or iron or sulphur, etc., it is clear, on theoretical grounds, that the oxidation or oxidations which take place must liberate heat. The demonstration of this fact, with reasonably small quantities of living organisms, has hitherto been nearly impossible. For this reason, it has been almost useless for the teacher to insist that the liberation of heat, the setting-free of energy, and not the material products, is the essential end, the fundamental characteristic of respiration.[2]

The apparatus of BONNIER,[3] which has furnished the most satisfactory results so far in the study of the heat-yield, is too elaborate and too costly for most botanical laboratories. On the other hand, the simple appliances described and figured in the laboratory manuals are useless, for they do not illustrate the subject, but mislead the student. Using the ordinary apparatus and the usual quantities of live and dead peas, for example, one obtains, with good luck, a difference of 0°.5 C. between the live and dead peas in the course of twenty-four hours, or perhaps a whole degree, or, best of all, 1°.5! This is mortifying enough, but if the temperature of the laboratory fall much, so that all the peas are chilled, there will be practically no difference at all. The evidence of the experiments, therefore, is all against the teacher who would have it that respiration is the means of supplying the living thing with the energy it needs to do its work. Evidently the trouble is with the insulation of the vessels in which the respiring and the dead plants or parts are contained; for if radiation and absorption were reduced to a minimum, the live and respiring peas would certainly grow warmer, while the dead peas would remain at the same temperature or grow slightly cooler.

[1] See BARNES, C. R., The theory of respiration. BOT. GAZETTE 39:81–98. 1905.

[2] See, for example, PEIRCE, G. J., Textbook of plant physiology, chap. 2.

[3] BONNIER, G., Recherches sur la chaleur végétale. Ann. Sci. Nat. Bot. VII. 18: 1893.

Convinced of this, as well as thoroughly dissatisfied with the insulating appliances which I had or could make in the laboratory, I went to my friends in the chemical laboratory of this university, told them of my difficulty, and asked for suggestions. Professor YOUNG, professor of physical chemistry, suggested trying some Dewar flasks. I take this opportunity to acknowledge my indebtedness, and to express my gratitude to Mr. YOUNG, for his suggestion is one which will be appreciated by any physiologist who tries the apparatus for this purpose.

Dewar glassware is made in several shapes—cylinders, cups, and flasks. The flasks are made either tubulated or with closed round bottoms. The Dewar apparatus is made also silvered or unsilvered. The principle is simple—double-walled vessels with the air between the two walls exhausted. Thus there is a receptacle surrounded more or less completely by a vacuum. Across this vacuum radiation or absorption will take place at a rate inversely proportioned to the perfection of the vacuum. If the walls of the vessel are silvered on the surfaces bounding the vacuum, the efficiency of the insulation will be greatly increased. The apparatus was devised for liquid-air experiments and is named for the inventor, the famous chemist at the Royal Institution in London. This double-walled glassware has now come into commerce and may be bought, under the name of "thermal bottles," in drug stores and of the dealers in automobilists' and campers' supplies. It is used for keeping food or drink warm or cold, as may be desired, for many hours. Thus soup, milk, coffee, ice-water, etc., can be maintained at the desired temperature for astonishing lengths of time. The commercial bottles are protected against breakage by cases of metal or basketry, but since these do not improve the insulation materially, they are unnecessary in the laboratory, and the thinner-walled scientific apparatus is much cheaper, besides being obtainable in a greater variety of shapes.

In the experiments which I am about to describe, I used silvered Dewar flasks of about 250^{cc} capacity, which were supplied by EIMER and AMEND of New York for $2.40 each. If imported duty free, as they would have been had I not been impatient to use them, they would have cost decidedly less. There should be at least two such flasks, for it is desirable to use always a lot of dead or other check material

for comparison; but it is naturally better to set up the experiment in duplicate and thereby reduce the sources of error. In many instances I used three flasks of live material and one of dead. Since the efficiency of each flask as an insulator depends upon the completeness of the evacuation of the space between the walls and upon the silvering it is evident that the flasks themselves will not be exactly alike, and that an average result is likely to be better than any single one. If proper pains are taken, the efficiency of each flask can be determined in advance; but unless the experiments are conducted in a constant temperature, as they should be, there is little use in doing this.

Miss BERTHA A. WILTZ, a graduate student and assistant in physiology in this university, did most of the actual work of setting up and recording the results of these experiments; but as we have worked constantly together, the experiments are ours rather than the work of either one of us. As the work progressed, experience showed us how the experiments should be improved in method, but the reasons for these improvements will be more evident if I describe one.

Experiment 1.—An unweighed quantity of dry peas was soaked for 24 hours in tap-water. They were then rinsed in boiled distilled water two or three times and divided into two unequal lots, the smaller of which was then covered with a fairly concentrated aqueous solution of corrosive sublimate for at least half an hour in order to kill these peas. The other lot was divided into equal parts, which were poured into two Dewar flasks of about 250^{cc} capacity, the one silvered, the other unsilvered. The dead peas were poured into another silvered flask of the same size. The flasks were cotton-plugged and suspended on strings (not wire), in such a manner that they would not touch any object, metallic or other. This was done to avoid the changes in temperature which might otherwise result. A thermometer reading only to degrees was pushed through the cotton so that the bulb was as nearly as possible in the center of each mass of peas. The data will be found in the accompanying table (p. 197).

Various things are evident in this first rough experiment. It was continued for nearly nine days and, in spite of the fluctuating temperature of the room, the temperature in the silvered flask containing live peas rose until the last day very steadily. Comparing this with

the temperatures recorded for the unsilvered flask, containing live peas, and with the silvered flask into which dead (killed) peas had been placed, one sees at once the superiority of the silvered flask as an insulator, and also that there was liberated and retained in the silvered flask containing live peas a very substantial amount of energy in the form of heat, even within the first twenty-four hours. Even the unsilvered flask gave a result better than I had ever been able to obtain with the ordinary insulators available in the laboratory.

Date	Temp. live peas silvered	Temp. dead peas silvered	Temp. live peas unsilvered	Room temp.
Feb. 26, 4:30 P.M.	17	17	17	....
27, 8:15 A.M.	19	16	13	....
12:15 P.M.	20	16	16	....
4:00 P.M.	23	17.5	19	....
28, 9:20 A.M.	32	15.5	15.5	16
12:00 P.M.	33	16	....	....
5:00 P.M.	36	16	20	....
29, 9:20 A.M.	38	15	15	15.5
12:30 P.M.	39	15	17.5	18
3:30 P.M.	40	15.5	19	17
Mar. 1, 10:30 A.M.	42.5	15	17.5	16.5
2, 10:20 A.M.	45	14	15	16
1:30 P.M.	47.5	14	18	19
5:00 P.M.	49	14.5	20	19
3, 9:25 A.M.	53	14	15	16
1:00 P.M.	54	15	19	....
4:15 P.M.	54+	15.5	22	22
4, —— A.M.	54.5	15	14	14.5
4, 2:30 P.M.	55+	15	23	18
4:30 P.M.	56	15.5	23	19
5, 9:15 A.M.	54	14.5	14	13
12:30 P.M.	54	....	....	....
6, 10:00 A.M.	50	14	14	....

When the experiment was stopped on the ninth day, because the temperature had begun to fall, it was at once evident that fermentation had been very active for some time. It was inevitable that this should be so, for I had not taken the slightest pains to sterilize anything. But in fermentation and decay heat is liberated, for these are processes in which respiration, as well as nutrition, takes place. We have here, then, the heat liberated in the respiration of the peas and of the other organisms in the flask. The obvious thing to do in succeeding experiments is to sterilize the peas and everything else used in the experiment, and to make similar experiments to determine the heat liberated by various ferment organisms. This I have done, to a

very limited extent and only as a preliminary, with Fleischman's "compressed yeast," as will be shown later.

This first experiment, with all its roughness, seemed of sufficient importance to repeat several times in order to be quite sure that there was no mistake about it. The results were similar in every respect, and I do not need to record them here. The following experiment shows the effect of some of the improvements suggested by the preceding.

Experiment 2.—Into each of six Dewar flasks of approximately equal capacity, which had been sterilized by being washed with a saturated aqueous solution of corrosive sublimate and rinsed with boiled distilled water (sterile), 80^{gm} of air-dry peas were put. The peas and the flasks were then sterilized by shaking the peas very thoroughly in the flasks with a 1:500 aqueous solution of corrosive sublimate, and this was rinsed off with two wash-waters, both sterilized. Fresh sterilized distilled water was then poured into the flasks and the peas soaked in them for twenty-four hours at a temperature which ranged from 20 to 22° in the oven in which I had placed the flasks. I did not take the temperatures in the flasks during this time, as my apparatus was not then so arranged as to make that possible. However, I shall repeat the experiment under constant temperature and with readings from the beginning. The data will be found in the accompanying table (p. 199).

In this experiment fermentation and decay were reduced very greatly, though perhaps not as completely as possible. Therefore we have a very fair index of the amount of energy in the form of heat which 80^{gm} of peas (weighed air-dry) can liberate in something less than three days. We also see that, in all probability, the efficiency of the individual Dewar flasks varies considerably. The efficiency of each flask should be determined and recorded. I have not done this because it would be useless unless pains were also taken to conduct the experiments in a constant temperature, and this I was not able to do at that time. I shall repeat the experiment under uniform conditions as soon as possible to arrange it. The thermometers used in this experiment were good ones, reading to tenths of a degree, loaned to me by the department of physiology of this university, and I take pleasure in thanking my friend Professor

Date	Time	Flask 1	2	3	4	5 dead*	6	Room temp.	Max.	Min.
Apr. 29.....	6:00 P.M.	22°7 C.	21°6	22°1	22°4	22°0	22°3	19°5		
Apr. 30.....	8:15 A.M.	23.7	22.3	23.8	23.8	18.2	23.0			
	12:15 P.M.	24.2	23.1	24.8	24.5	18.3	24.0	20.5		
	5:30 P.M.	27.0	25.7	27.3	27.4	19.6	26.5	21.0		
May 1.....	8:15 A.M.	31.1	31.7	32.4	32.4	17.4	31.3	14.5		
	12:30 P.M.	32.3	33.5	33.9	33.8	17.7	33.0	18.2		
	eve.	33.4	35.1	35.3	35.0	18.2	34.5	19.0	27°5	14°4
May 2.....	8:45 A.M.	35.7	38.4	39.0	37.5	17.6	37.5			
	12:30 P.M.	36.2	39.5	40.2	38.5	18.0	39.0			
	3:50 P.M.	36.8	40.5	41.4	38.8	18.4	40.0	18.3	26.2	16.1

Experiment stopped at 3:50 P.M., May 2.

	Flask 1	2	3	4	5	6	
No. peas decayed†.	3	2	4	2	0	3	Radicles 2.5–3^{cm} long.

Some of the sprouted peas planted in soil all grew normally.

* The peas marked *dead* were killed as in experiment 1.

† These decayed peas were without exception weevily, and because of the boreholes of the weevils could not be sterilized. Since the weevils close the outer ends of the holes, it is impossible to detect all the weevily peas when working with considerable quantities.

MACFARLAND for his help in this particular respect as well as in others. Owing to the position of the thermometers, making uniform sighting impossible, I do not record the attempt to read to fractions of tenths of degrees.

To see whether the heat liberated by a small amount of yeast in a small volume of a fermentable solution could be measured by the method described in the foregoing pages, I carried on some experiments, only one of which I need to report now. The yeast used was "Golden Gate compressed yeast," which is, I believe, only one of the many local names for what passes in the east under the name of Fleischman's compressed yeast. Four flasks were sterilized by being washed thoroughly with a saturated aqueous solution of corrosive sublimate. They were subsequently rinsed twice with sterilized water and plugged with cotton. Into each of these flasks 250^{cc} of 10 per cent. solution of cane sugar were poured as quickly as possible. The solution had previously been sterilized in four cotton-plugged flasks, from each of which it was poured into a Dewar flask. Since the yeast to be added is by no means a pure culture, I thought this sufficient care to exercise with the solution. To three of these Dewar

flasks approximately 4.5^{gm} of a yeast cake were added, to the fourth flask nothing. The yeast had been quickly rubbed up in a sterilized mortar, with a sterilized pestle, in a small quantity of the sterile sugar solution. Thus, on shaking the flask after adding the yeast, I hoped to mix the yeast thoroughly with the whole volume of fermentable liquid. The fourth flask contained, then, only sterilized sugar solution, which had been exposed to the air for only a moment in transferring it from one sterilized vessel to another. The data follow.

Date	Time	Flask 1	2	3	4	Room temp.
					(no yeast)	
April 8.....	6:00 P.M.	19°.1 C.	18°.6	18°.6	17°.8	
April 9.....	9:15 A.M.	22.1	20.5	21.1	17.5	
	5:30 P.M.	23.3	21.5	22.3	17.7	20°.0
April 10.....	8:00 A.M.	24.2	20.6	23.0	17.2	15.3
	12:30 P.M.	24.5	20.8	23.4	17.2	19.5
April 11.....	8:15 A.M.	25.0	20.6	24.0	17.3	
	12:30 P.M.	25.1	20.7	24.2	17.4	20.0

At this point, two days and eighteen hours after the experiment was started, it was ended and the flasks opened. The odor and flavor of the solutions were pleasant. I used the same thermometers as in the preceding experiment, but for the reasons previously given record here nothing less than tenths of degrees. Of course the thermometers were sterilized, by standing in saturated corrosive sublimate solution, and afterward washed in sterile water, before being introduced into the flasks. The efficiency of a good Dewar flask as an insulator is indicated by flask 4 in this experiment; for although the temperature of the room varied at least 5° C., as the record shows, the temperature within this flask varied only 0°.6 C., according to the readings taken at the same times. Whether flask 2 was a poor one, or whether the yeast was poor, or what the trouble was, I do not know. In the other two flasks the mercury rose to a degree which surprised me, considering the small amount of yeast sown in each of the three flasks and the small volume of fermentable liquid. This rise in temperature indicates the liberation of considerable energy in the form of heat.

In connection with these experiments on the respiration of healthy

plants I made one experiment, purely preliminary like the others, on wounded plants. RICHARDS reported in 1896 and 1897,[4] as a result of his experiments, that plants develop fever on wounding, as animals do. This increase in temperature is due to increased respiration in both sets of organisms. RICHARDS' methods are excellent, but unless an efficient insulator is used or the experiments are carried on in a constant temperature, they are not absolutely exact. I determined, therefore, to try Dewar flasks in a simple experiment of this sort. Two lots of onion sets (seedling onions) were carefully skinned, thus removing much if not all of the dead tissue by which these young bulbs are surrounded. Each lot was found to weigh 111^{gm} after skinning. One lot was put whole into a sterilized flask (no. 2), care being taken that the onions were not scratched or bruised in being put into the flask. The other lot was cut into irregular pieces, each onion into four to eight pieces, with an ordinary and fairly dull knife and put into another flask (no. 1). The temperature record follows.

Date	Time	Temp. no. 1 (chopped)	Temp. no. 2 (whole)	Room temp.
April 26.....	2:00 P.M.	17°.5 C.	17°.50	17°.0
April 27.....	10:20 A.M.	23.25	18.50	19.0
	1:30 P.M.	25.00	19.50	....
	5:00 P.M.	27.00	20.25	20.0
April 28.....	12:15 P.M.	38.00	20.50	....

The experiment was stopped at noon, when it had run nearly two days, and the contents of each flask turned out upon the table. Both lots of onions appeared to be in perfectly normal condition. In some cases the edges of the cuts of the wounded onions were a little dry. The material looked as if the experiment could have been continued for twice this length of time without decay or other disturbance setting in. It would seem, therefore, that these flasks can be used for such experiments on wounded plants.

In the experiments here reported, temperatures are given as the evidence that energy (heat) is liberated in respiration. Although these temperatures are interesting, they do not give us any idea of the

[4] RICHARDS, H. M., Respiration of wounded plants. Annals of Botany 10:1896; 11:1897.

amount of heat liberated by a given organism or part. Because of the roughness of these preliminary experiments, and of my lack of the apparatus for carrying on the experiments under constant conditions, I have made as yet no effort to ascertain the number of calories liberated by a given weight of germinating peas. I hope to do this presently, not only for peas, but also for other things; but I do not wish this statement to be taken as suggesting that I wish to keep this method at present for my own use.

Dewar flasks seem to me to offer to the physiologist, both animal and plant, a convenient means of testing the yield of heat by respiration, testing in the case of an animal the calorific value of its food, testing in plants and plant parts the liberation of heat at various stages and under various conditions. Since the Dewar glassware is obtainable in various forms, and can be made in others if desired, it can be used for all the purposes of BONNIER'S experiments and for many others. For example, I see no reason why it would not be possible to ascertain the respiratory curve of any particular plant, from the beginning of the germination of its seeds until it had attained considerable size; to ascertain more exactly than we now know the relation of respiratory activity to the other activities or to the stages of development of the plant. These flasks, or cylinders, can be used also for demonstration experiments, on the lecture-table for example, proving at once to a class that respiration is really a process in which energy is released, and that it is the chief process by which the living organism obtains the energy which it constantly needs and uses.

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THE SEEDLING OF CERATOZAMIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 115

HELEN A. DORETY

(WITH TWO FIGURES AND PLATES XII–XVI)

The vascular anatomy of adult cycad stems is likely to be obscured by two sets of complications. One, called anomalous thickening, described by VON MOHL (**14**) in 1832 in species of Encephalartos, is limited to certain forms; the other, the phenomenon of girdling, was described by KARSTEN (**5**) in *Zamia muricata* Willd. in 1856, and by METTENIUS (**8**) in Cycas, Encephalartos, and Dioon in 1860. Since then it has been found in the three other genera investigated. In the seedling these complications are either absent altogether or are just being initiated, hence the selection of juvenile forms is probably even more important in the anatomical study of cycads than in that of any of the other vascular plants. Anatomists have recognized this and have given us numerous descriptions, only the more pertinent of which need be cited here.

In 1856 KARSTEN (**5**), in the work mentioned above, found that each of the cotyledons of Zamia receives only one bundle, which may branch; that the central cylinder is broken up by parenchymatous communication between pith and cortex; and that in older stems, with the increase in the number of cauline strands, there is a corresponding increase in the number of bundles entering the leaves, because every bundle of the central cylinder contributes a trace. He also observed the frequent anastomosing of the leaf traces reported by VON MOHL, as well as the occurrence of girdling.

In 1884 BOWER (**1**) wrote a few descriptive notes on the seedling of *Cycas Seemanni* Al. Braun. He found the two cotyledons unequal in size and dissimilar in vascular anatomy. He says that while in some there is a median bundle which extends the whole length of the cotyledon without branching, in others there are two equal bundles near the center.

In 1887 GREGG (**4**) reported anomalous thickenings in the root of this species.

In 1896 WORSDELL (**16**) discovered them in the adult stem of *Macrozamia Fraseri* Miq., giving it an appearance which recalled to his mind the stem of the Medullosae.

In 1897 the same author (**17**) gave the name "transfusion tissue" to certain short, scattered tracheids with reticulate markings on their transverse walls, found in lateral communication with the bundles in the green parts of some gymnosperms. He considers this tissue a "direct derivative of the centripetal xylem" in the vascular bundles of fossils. Later in the same year (**18**) he recorded its occurrence in the cotyledons of *Cycas revoluta* Thunb. and in the leaf traces of *Stangeria paradoxa* T. Moore. He found that in each of the two cotyledons of *Cycas revoluta*, there are three collateral bundles, which may increase to five; that there may be some centrifugal wood near the base of the cotyledon, but none farther out; that the root may be tetrarch or triarch; that girdling had not yet begun in the young seedling he was investigating; and that anomalous thickenings were conspicuous. In *Stangeria paradoxa*, the two cotyledons have a common stalk, each is multifascicular, and the bundles are said to be concentric near the base. The root is triarch, changing to diarch near the tip. In the same paper, the two cotyledons of *Macrozamia spiralis* Miq. are said to be like those of *Cycas revoluta*.

The author calls attention to the absence of anomalous thickenings in this species of Macrozamia in contrast with the mature stem of *M. Fraseri* previously studied by him. In all three seedlings WORSDELL missed the transition from stem to root.

In 1898 the seedling of *Bowenia spectabilis* Hook. was described by PEARSON (**9**). He says that each of the two cotyledons has four to seven bundles derived from one, and that these bundles are all collateral with normal orientation. He is emphatic as to the collateral nature of the bundles, even after having examined a preparation of WORSDELL'S in which the latter considered them concentric. In the leaves the bundles are oriented normally, and the centripetal wood, scanty at the base, becomes more and more abundant farther up the petiole; but the centrifugal wood does not disappear even in the pinnae. The root is tetrach or pentarch, but may reduce to triarch. In the young material at his disposal, PEARSON found no anomalous thickenings in the root, but they were discovered later in presumably

older plants by WORSDELL (**19**), who seized the occasion to emphasize the similarity to the Medullosae.

In 1904 MATTE (**6**) published his masterly thesis, which in the third part treats of the seedlings of *Dioon edule* Lindl., *Cycas siamensis* Miq., and *Encephalartos Barteri* Carruth. Of his two seedlings of *Dioon edule* one proved abnormal, one cotyledon being partly aborted. The cotyledonary bundles are mesarch near the base, but the protoxylem moves quickly outward, so that the bundles are exarch throughout most of their course. The axis is considered as an aggregation of leaf bases, and the girdling is explained as being due to intense intercalary growth produced under the influence of the developing leaf within. The four root poles are inserted upon the cotyledonary bundles, the median bundles furnishing insertion for two diagonally opposite poles, and each lateral trace uniting with its mate of the other cotyledon to furnish insertion for the other two poles. Mucilage canals are reported in the root, for the first time in a cycad. The root of *Cycas siamensis* is diarch; its two poles are inserted upon the median bundles of the cotyledons, the lateral bundles dying out in the cortex. Anomalous thickenings or cortical vascular strands are conspicuous; MATTE'S demonstration that these are not abnormal, but merely remnants of an ancestral character, justifies his objection to the term "anomalous" used to describe them.

In Encephalartos the root is pentarch, and the cotyledonary bundles—a single, ringlike trace from each of the three cotyledons—fuse with the leaf traces at different levels before entering the central cylinder. This cylinder is polystelic, as in *Medullosa anglica* Scott. In considering this feature, MATTE indorses WORSDELL'S view that it helps to relate the Cycadales to ancient forms like Medullosa rather than to monostelic forms like Heterangium as SCOTT suggests. He regards the root of cycads as a "new organ" inserted upon the lower extremity of the hypocotyl, and not merely an extension of that organ.

The main facts discovered by these investigations, and agreeing, so far as they relate to *Dioon edule*, Cycas, and Zamia, with unpublished studies made in this laboratory by LAND, THIESSEN, and others, may be classified as follows:

Characteristic features.—Under this heading may be placed the occurrence of two cotyledons, the hypogean character of germination, the thick tap root, centripetal wood in the cotyledons and leaves, girdling, and numerous mucilage ducts.

Character upon which the seedlings may be divided into two groups.—This is the presence or absence of anomalous thickenings or cortical vascular strands. They occur in Cycas, Bowenia, and Encephalartos; they are said to be absent in Zamia, Dioon, Macrozamia, and Ceratozamia, though they were found in the mature stem of *Macrozamia Fraseri* by WORSDELL. Microcycas remains to be reported upon.

Features peculiar to certain genera.—We may place here the single cotyledon reported for Ceratozamia, the three cotyledons of Encephalartos, concentric bundles in the base of the cotyledon of Stangeria, the polystele in the earliest formed part of the axis of Encephalartos, and mucilage ducts in the root of Dioon.

The present series of studies aims in the first part to add to the list a detailed account of Ceratozamia, Microcycas, *Dioon spinulosum*, and species of Zamia; and in the second part to extend the investigation to the conifers by examining some juveniles of each of the great groups. Podocarpus, Keteleeria, Cunninghamia, *Pinus edule*, *P. Banksiana*, and a few others, as well as the cycads mentioned, are well under way.

Ceratozamia

In 1846 BRONGNIART (**2**) gave the generic and specific names to *Ceratozamia mexicana*, a "new cycad from Mexico." He described the adult forms and the ovulate and staminate strobili. In 1870 WARMING (**15**) reported the monocotyledonous character of the embryo. In 1872 VAN TIEGHEM (**13**) examined four seedlings and found a thick scale enclosed by the sheathing base of the single cotyledon, and, in turn, enclosing a hairy foliage leaf with circinate vernation. One of the four seedlings was suspected by him of having the rudiment of a second cotyledon. He describes the root after the xylem and phloem have reached their final position, and gives the number and derivation of the cotyledonary strands and early leaf traces; but in these respects, no two of the seedlings agree. A piece of mature stem, examined by SOLMS-LAUBACH (**12**) in 1890, was

found to have a single cylinder, but peduncular strands occurred in the pith, an observation which was corroborated later by WORSDELL (**16**). In the work previously mentioned (**6**) MATTE made a careful study of the ovulate cone and the mature leaf, with a few observations on younger leaves. He found terminal teeth on some of the pinnae of the younger plants, and at the bases of the petioles stipules with a bundle in each. He says that the meriphyte of the leaves has a modified Ω-shape in cross-section, with an anterior system, and shows that in this as well as in other cycad petioles the so-called fusions of the traces are often only approximations (*accolements*).

MATERIAL AND METHODS

The seedlings of Ceratozamia and *Dioon spinulosum* were grown from seeds provided by Professor CHARLES J. CHAMBERLAIN; the Microcycas seedlings were given to me by Professor OTIS W. CALDWELL, and later some were grown from seeds secured by Professor CHAMBERLAIN. The Zamia seedlings were furnished by Dr. W. J. G. LAND.

Having in mind the danger of drawing conclusions from a few specimens, I have used freely the wealth of material at my disposal. The chief part of the investigation was made from material fixed in picro-acetic-alcohol, stained with safranin and anilin blue, and cut in serial sections. *Figs. 21*, *22*, and *23* are diagrams of wax models constructed from serial sections.

GENERAL DESCRIPTION OF EMBRYO AND SEEDLING

The seeds of Ceratozamia are about 2.5^{cm} long and 1.5^{cm} thick, that is, about the same size as those of *Dioon edule*, and intermediate between *Dioon spinulosum* on the one hand and Zamia on the other. Like most cycad seeds, they are flattened on two or three sides by the pressure of growth within the cone, and it is not unimportant to remember that during the whole period of embryonal development —the longest known for a cycad—the seed lies upon whatever side it happens to fall. The single cotyledon begins to be differentiated in November, and early in December its appearance is like that represented in *fig. 1*. Later it begins to surround the axis (*fig. 2*), and finally the two edges meet (*fig. 3*). In these early stages, the coleorhiza is proportionally long; but later elongation of the coty-

ledons forces the base of the embryo backward, so that both coleorhiza and suspensor are crushed little by little to a flat, brown disk (*figs. 4, 5*). Further elongation forces the base of the embryo through the softer, micropylar portion of the seed coat; *figs. 6, 7, 8* show this and the two succeeding stages. In *fig. 7* the base of the cotyledon is curved downward, and the plumule is seen issuing from between its opened edges; and it may be observed that this method of development has thrown the first leaf out of alignment. The edges close in again, and remain adhering the full length of the cotyledon (*fig. 13*). The tip is sometimes lobed (*figs. 14, 15*).

The cotyledon always develops on the lower side of the embryo as the seed lies during germination. In seedlings which were turned after the cotyledon had begun to develop, the plumule has not succeeded after a year in emerging from underneath the cotyledonary sheath with which it is hampered.

Fig. 8 shows the appearance of the tardy root, which has made its way through the brown cap formed from the remains of the disorganized coleorhiza and suspensor. When the root pierces the soil, the starch is transferred to it from the endosperm, and the root thickens into a tap root. By its further penetration into the soil, it often draws the upper portion further down, imbedding the seed, and possibly giving to the first series of lateral roots their initial upward slant. The lateral roots almost always appear in threes, whether the root be tetrarch or triarch. *Fig. 9* represents a seedling toward the close of this period of its activity. The extreme shortness of the hypocotyl may be conjectured from the small distance between the base of the cotyledon and the insertion of the first whorl of lateral roots. The plumule is composed in this case of two brownish, hairy scales, enclosing a foliage leaf with circinate vernation (*fig. 10*). Each scale is terminated by a sharp, curved point. The number of scale leaves varies in different seedlings; some have only one, and in in some few observed the first organ was a perfect foliage leaf. The base of both scale leaf and foliage leaf is furnished with broad, wing-like expansions which enclose the next leaf. The petiole of the first leaves reaches a length of 15–20^{cm}, and bears one or two pairs of pinnae inserted near the adaxial face. Between the two terminal pinnae there is a tiny, sharp spine, which has its counterpart in the

apical point of the scale leaves. At irregular intervals along the petiole are other, even smaller points. The first leaves are opposite, but the later ones assume the spiral phyllotaxy represented in *fig. 12*.

ANATOMY

As was above noted, the edges of the cotyledon close in after the exit of the plumule, the two halves of the adaxial face meeting in a plane represented by the line *ad* in *fig. 13*. Three bundles enter the cotyledon from the vascular plate, and each dichotomizes again and again (*fig. 16*), the median one being no more a "double" bundle than any of the others. The number of traces may be increased to fifteen toward the upper portion, but is gradually reduced again toward the tip, some bundles approximating in pairs, others dying out. Those in the lobes disappear lower down than the others (*fig. 15*). The bundles are collateral throughout the cotyledon, and their orientation is normal, as seen in *figs. 13, 14, 15*, where the xylem faces the line *ad*. The wood is mesarch (*fig. 17*), with the protoxylem gradually moving out toward the phloem as it ascends. In the upper portion centrifugal elements are wanting, that is the wood is exarch (*fig. 18*). The vascular system of the cotyledon is differentiated relatively early in the development of the seedling. In that from which *fig. 19* was drawn, in which no root has yet appeared, the vessels are fully matured. WORSDELL'S transfusion tissue was frequently observed in direct continuation with the centripetal xylem (*fig. 18, tr*).

Mucilage canals are numerous in the lower part, and seem to bear no definite relation to the strands (*figs. 30, 31*); farther up they usually number one more than the strands and alternate with them; higher still they die out irregularly; in some cotyledons they are absent altogether.

The vascular plate of the hypocotyl axis is irregularly four-sided as seen in cross-section; all the xylem is in the center, sometimes in a solid mass, sometimes interspersed with pith cells. The protoxylem points are clearly distinguishable. In the plant from which *fig. 20* was drawn, the protostelic condition persisted through a vertical distance of 1.6^{mm}. Above this the pith cells occupy the central region, so that a siphonostele replaces the protostele (*fig. 28*). About

1^{mm} above the upper terminus of the protostele, the xylem is grouped sometimes in four conspicuous mesarch lobes, but oftener in three prominent ones and a fourth weaker one (*D*, *fig. 29*). From this weaker lobe a very small strand passes out (*D*, *figs. 21–24, 26, 30*), and in some cases it branches, but is lost in the cortex. From the lobe diametrically opposite, the median strand of the cotyledon (*C*, *figs. 21, 24, 25, 29, 30*) enters that organ; and from each of the other two angles or lobes a strand passes out (*A*, *B*) and branches, one member in each case bending tangentially (*b*, *b'*) to form the lateral trace of the cotyledon, the other, a very small strand (*a*, *a'*), either fusing with the leaf traces or dying out in the cortex. This cotyledonary node is represented in cross-section in the diagram (*fig. 24*).

Comparison with the same node in Zamia, Cycas, *Dioon edule*, and Microcycas suggested that the smaller bundles (*D*, *a*, *a'*) were the mates of the larger ones on the side of the cotyledon (*C*, *b*, *b'*), and that the second cotyledon was suppressed. The cause of the suppression was indicated by the long-continued one-sided presentation to gravity during germination, and the fact that the cotyledon is always on the under side. It was with the intention of testing these surmises that the experimental work already recorded (**3**) was undertaken. *Fig. 25* represents the cotyledonary node in Ceratozamia embryos developed on the clinostat, and *fig. 13a* a transverse section of their cotyledons.

Although the stem contains several layers of extrafascicular cambium (*cb*, *fig. 32*), I have not been able to find in two-year-old plants any anomalous thickenings except the solitary bundle (*fig. 33*) whose position in the base of the cotyledon is indicated in *fig. 30* at *z*. This bundle has its origin in a small group of cells (*cb''*, *fig. 32*) in the outermost layer of cambium. It is about 0.4^{mm} long and approximately vertical. Its tracheids have only spiral thickenings.

The foliar bundles (*fb*) may occur in four groups alternating with the cotyledonary bundles (*figs. 21–23, 26, 30*); but oftener there are only three groups, because of the fusion of two of them or the entire elimin tion of one, on the side of the suppressed cotyledon, when the main bundle (*D*) is slight or entirely wanting (*fig. 26*). At first they are all vertical; higher up they branch, and a strand from each group

is sent to the first leaf (*fig. 22*). As previously noted, this organ, whether scale leaf or foliage leaf, is displaced laterally by the peculiarities of its development. All the traces which enter it are likewise distorted, giving rise to a pseudo-girdling condition which is apparent in very young seedlings (*figs. 22, 27, 31*). The two traces which enter from the groups nearest the leaf (*e, e'*) take first a radial, and then a tangential course to reach a position in the middle of the leaf (*figs. 22, 31*), showing on the way a tendency to branch. The traces (*d, d'*) supplied from the groups farther away take a tangential course, each giving off vertical branches, which in turn branch again. The remaining traces of the original four foliar bundles ascend vertically (*fig. 25*), and branch and anastomose freely. Only a limited number, in most cases four or five, remain (*jb, figs. 24, 31*). Before reaching the leaf base, each of these divides, one member entering it (*t, fig. 23*), the other, which remains small, being directed toward the growing point of the stem (*u, figs. 23, 27*). *Fig. 23* represents the branching and anastomosing of these strands. It should be noted that the vertical scale of the three diagrams (*figs. 21–23*) is magnified considerably.

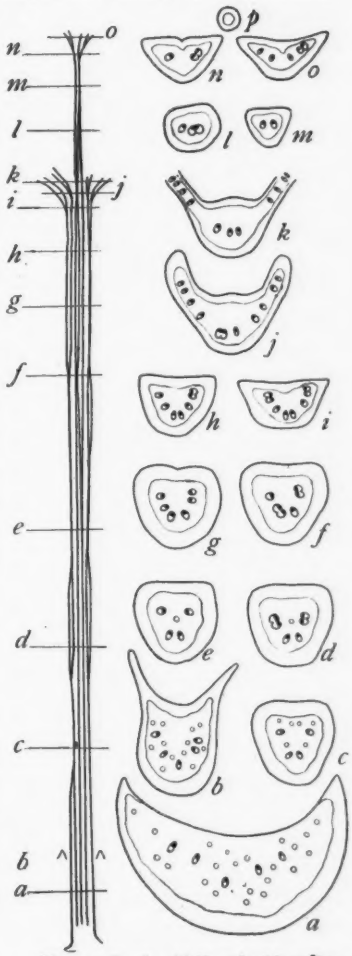

FIG. 1.—Study of foliage leaf from base (*a*) to apex (*p*); for explanation see text.

In the younger stages there is no attempt at girdling on the part of the leaf traces, except that which has been referred to in the first

leaf as pseudo-girdling, whose cause is to be sought in the accident of its development. When the inner face of a leaf encloses a mass whose diameter is less than 3^{mm} (fourth leaf in *fig. 12*), the vascular bundles of that leaf are all vertical; but when the enclosed mass, by its enormous radial growth, has reached a diameter of 5–8^{mm} and comprises two or more developing leaves (second leaf in *fig. 12*) the base of the enclosing leaf enlarges its inner face accordingly. The first stage in the enlargement consists in an increase in the number of cells, but the second in a horizontal elongation of these (*text fig. 1*). While this is going on the older part of the central vascular cylinder is also increasing its diameter, separating farther and farther the original positions of the bundles of the leaf. As a result, the marginal traces gradually elongate as they are drawn more and more from the vertical position, and their upper parts stretch outward in the direction which the leaf takes. These facts have been observed repeatedly; whether they are an adequate solution of the problem of the cause of girdling, as MATTE thinks, I am unable to say.

In seedlings with three or four leaves, the stem bundles (*u*, *fig. 19*) branch repeatedly, and many of the branches reunite to form a small number of traces. Each of the remaining ones now forks once, the larger member in each instance going to the leaf as before, the smaller one continuing in the axis. Thus, even at this early stage, there is present the sympodial stem described by Miss SMITH (**11**) for older plants.

The number of strands entering successive leaves was seen to increase, sometimes with great regularity. In the plant whose dissection is represented in *figs. 11* and *12*, the number of traces was increased by one in each successive leaf, from the cotyledon with three, to the fourth leaf with seven; but the increment was not so constant in all the plants observed.

Within the leaf base and in the petiole, the bundles branch and anastomose freely. There is no real Ω in these first leaves; the bundles are arranged simply in an open arch. *Text fig. 2* is a study of a foliage leaf which was the second lateral organ of the plumule, that is, it was preceded by only one scale leaf. The scale leaf had four bundles; this leaf has five (*a*, *b*); but just above the stipules the number is reduced to four by the approximation of two of them (*c*).

Other changes occur as the meriphyte ascends the petiole (*c*, *d*). Just below the first pair of pinnae the branching is rapid, and at the level where the blade is seen exteriorly to separate from the petiole, there are four strands provided for it (*j*). In the rachis another approximation takes place (*l*), and the number of bundles is reduced to two (*m*). Each enters a pinna and branches continuously (*n*, *o*). The growing point terminating the rachis has no vascular trace in this leaf, in this respect agreeing with the young leaf represented in *fig. 10a*. As the plant increases in age, the meristem of this point retains its activity longer, producing a greater number of pinnae, as in the seed plants.

The wood in all the leaf traces is endarch in the central cylinder (*fig. 26*), but it soon becomes mesarch, with the protoxylem moving outward by almost imperceptible degrees. It never reaches the exarch condition, however; even in the tips of the pinnae there are always two or three elements of centrifugal wood. Here and there may be seen a few elements of transfusion tissue.

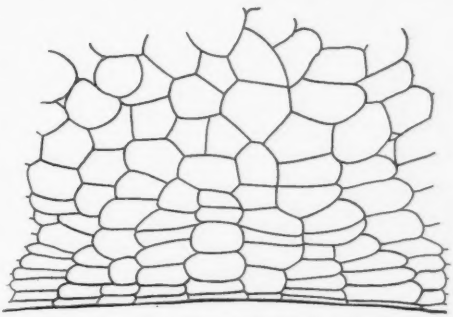

FIG. 2.—Cross-section of flattened cells from inner face of petiole near the base.

Mucilage canals are numerous in the leaf bases (*figs. 30*, *31*), but they have no more definite arrangement there than in the base of the cotyledon. Throughout the region of the stipules there is a gradual decrease in number, which is not altogether due to fusing. Toward the upper levels of this region they are sometimes arranged in two series, an adaxial row and an abaxial one, with a definite relation to the bundles (*text fig. 2b*). About 1^{cm} above the base, there is left only one centrally located canal (*d*), which persists for half the length of the petiole (*e*).

The bases of the leaves as well as of the cotyledons are covered by a thick layer of cork. As was seen externally, the hypocotyl is extremely

short, the transition from stem to root taking place in an almost horizontal plane (*fig. 30*). In the absence of cauline bundles, the root poles are inserted upon the cotyledonary strands (*A, B, C, D, figs. 34, 35*). There is no rotation of the protoxylem, but the phloem and metaxylem separate opposite the protoxylem groups (*Bn, Cn, fig. 36*), the cambium proliferating to fill the breach in the metaxylem, and the cortex invading the space left vacant by the phloem. The resulting halves swing, the one to the right, the other to the left, giving the appearance of a double fan of phloem and metaxylem connected by a single group of protoxylem. The right half of the phloem of one pole joins the left half of that of the next, and the cambium layer is thus curved inward (*cb, figs. 37, 38*). One result of this complicated process is that the entire xylem system of the root is bordered peripherally by cambium.

The medulla throughout the root is extremely meristematic, and its activity sometimes results in a displacement of one or more of the protoxylem groups, giving an unsymmetrical appearance in cross-section. This activity is indicated by the thin walls (*o, fig. 38*) showing recent division.

In all the plants I have investigated, the number of poles remains constant throughout any given root. This number may be three or four, depending seemingly upon the degree of development attained by the median bundle of the aborted cotyledon. *Figs. 26* and *34* will serve to illustrate this point. They represent the same level in two different plants. In the former the median bundle (*D*) was very weak, and there were but three root poles; in the latter this bundle was well developed and the root was tetrarch.

Fig. 39 represents the condition of the root tip in the region of the differentiation of protoxylem. The connective tissue is clearly defined, but there is no xylem plate connecting the poles. Examination of longitudinal sections of the root tip furnishes nothing that could be added to the description of REINKE (**10**). One initial group produces plerome, another periblem. There is no calyptrogen or dermatogen, but the outermost layers of the periblem become loosened at the tip and form the root cap (*k, fig. 42*).

There is no distinct endodermis, and the pericycle is several-layered, making it impossible to distinguish with absolute cer-

tainty any line of demarkation between phloeoterma and stelar tissue.

There are no anomalous thickenings in the roots of plants two years old.

In general, mucilage ducts extend but a slight distance into the root, usually about 8^{mm}, but in one specimen they were beginning to be formed in the plerome, immediately behind the region of its differentiation (*md, fig. 42*).

The lateral roots are diarch. *Fig. 40* represents a transverse section of a secondary root and the exit of a tertiary one. The uppermost lateral roots become ageotropic at a very early stage, and show symptoms of bacterial infection; and in some of the two-year-old plants, these roots present the characteristic "coralloid" appearance indicative of algal infection.

The root, as was seen above, is late in developing. *Fig. 27* represents the position and form of the meristematic plate, which has given rise to stem above, but is yet inactive below. *Fig. 19* shows the beginning of the activity which produces the root; the central portion of the active region in this figure is sketched in greater detail in *fig. 41*. In the stage represented in *fig. 42*, the root cap is developed and the plerome and periblem initial groups are easily distinguishable, but the plerome has not yet differentiated any xylem elements.

While in older seedlings the vascular systems of stem and root seem to be continuous, one sometimes finds sections which indicate a certain amount of interruption. Such a case is shown in *fig. 43*.[1]

Discussion

The result of the experimental work on Ceratozamia places this genus in line with the other cycads with reference to the dicotyledonous

[1] When this investigation was completed, MATTE (7) published a preliminary note on the same subject. He says that the petiolary bundles have the Ω arrangement, that there is an anterior fascicular system in the region of insertion of the rachis, and that the pinna has terminal teeth. These features do not appear in any of my seedlings. Speaking of the root, he says it is tetrarch, and that he has observed a progressive reduction of poles, to three and even to two. As I have observed, no reduction occurred in the roots of any of the seedlings I sectioned, and the root is triarch as often as it is tetrarch. He has also found the abnormal thickenings strongly developed; but he does not give the age of the seedlings which manifest them.

character. The proof of the abortion of the second cotyledon and the discovery of the manner in which that abortion was brought about naturally revive the question of the monocotyledonous nature of certain dicotyledons. It is possible that in some cases this condition may be caused by the same factor which produces it in the embryo of Ceratozamia, but this is not so in all cases; the experimental work now in progress upon these forms shows that other factors are involved.

The lobing at the tip of the cotyledon is suggestive of a primitive condition, which will be discussed in connection with *Dioon spinulosum*.

It would be interesting to compare the cotyledonary node in all cycads, in order to determine whether they are modifications of the same type or whether there are different types; but it is a matter of regret that all investigators have not considered it of sufficient importance. MATTE has described it fully for *Dioon edule* and *Cycas siamensis*. The latter is clearly a modification of the type which may be represented by the former. The present paper shows that Ceratozamia conforms to this type, and it may be in place here to say that Microcycas and the species of Zamia I am investigating are the same. WORSDELL'S *Cycas revoluta* and *Macrozamia spiralis* are doubtless similar. *Encephalartos Barteri* according to MATTE'S description, *Zamia muricata* according to KARSTEN'S, and *Bowenia spectabilis* according to PEARSON'S seem to differ from this type, in receiving only one bundle from the central cylinder; but there are two facts which conspire to make us consider that the seedling of Encephalartos described by MATTE was an unusual one: the presence of three cotyledons, and the union of the cotyledonary strands with the central cylinder at different levels.

That cycads, especially such fernlike ones as Stangeria and Bowenia, should be found to have an occasional concentric bundle is only to be expected from the nature of their fern origin. WORSDELL'S announcement, then, of such bundles in the base of the cotyledon of Stangeria was not a surprise, even though his drawings were not convincing. But the emphatic statement of PEARSON that he could not be convinced of it in WORSDELL'S preparation of Bowenia raises the doubt whether he would have recognized it in Stan-

geria. Of course the whole question resolves itself into the possibility of determining phloem in the absence of sieve plates.

In the feature of anomalous thickenings, as in so many other features, Ceratozamia appears to hold an intermediate position. The extrafascicular cambium is clear and distinct, yet in plants with two scale leaves, two expanded foliage leaves, and two or three leaves developing, the secondary fascicular systems are entirely wanting. The failure or delay of this cambium to function indicates that it is a vestigial character. I have seen it in Zamia as clear and distinct, though not so abundant, as in Ceratozamia.

There seems to be great variation in the number of root poles throughout the whole group of cycads and even in individual roots. This variation, taken in conjunction with the fact that in Ceratozamia the number depends upon the degree of development of the median bundle of the aborted cotyledon, indicates that this character is not to be depended upon as a phylogenetic one.

Summary

1. Ceratozamia is dicotyledonous, the second cotyledon being aborted by gravity.

2. The cotyledon is often lobed at the tip. It is multifascicular, and all the bundles are derived from three. The wood is mesarch at the base and exarch in the upper portion. Mucilage ducts usually alternate with the bundles.

3. The leaf traces are at first vertical; girdling follows upon increase in radial growth of the enclosed leaves and stem apex. The wood of the leaf traces is endarch in the central cylinder, but becomes mesarch in the leaf base and remains so to the tips of the pinnae.

4. The scale leaves are aborted foliage leaves.

5. The first-formed portion of the central vascular cylinder may be a protostele.

6. The stem is a sympodium.

7. There are several layers of extrafascicular cambium, but in seedlings two years old only the slightest trace of anomalous thickening.

8. The root is a delayed organ, and its four poles are inserted upon the cotyledonary bundles.

9. The entire xylem system of the root is bordered peripherally by cambium.

Grateful acknowledgments are due to Professor JOHN M. COULTER and Dr. W. J. G. LAND, under whose direction the investigation was conducted, and to Professor CHARLES J. CHAMBERLAIN for abundance of material.

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EXPLANATION OF PLATES XII-XVI

All the drawings except *figs. 6–11, 16, 21–25* were made with the aid of a camera lucida. The abbreviations used are as follows: *A*, *B*, *C*, *D*, the four main cotyledonary bundles; *An*, *Bn*, *Cn*, *Dn*, the corresponding root poles; *C*, median bundle of developed cotyledon; *D*, median bundle of aborted one; *a*, *a′*, lateral bundles of aborted cotyledon; *b*, *b′*, lateral bundles of developed one; *ad*, adaxial face of cotyledon; *c*, cotyledon; *cb*, cambium; *cb′*, *cb″*, extrafascicular cambium; *cl*, coleorhiza; *ct*, connective tissue of root; *cx*, cortex; *cfx*, centrifugal xylem; *cpx*, centripetal xylem; *d*, *d′*, lateral traces of first leaf; *e*, *e′*, middle traces of same; *f*, foliar bundles; f^1, f^2, f^3, f^4, four principal groups of foliar bundles; *g*, cotyledonary trace; *h*, vascular system of hypocotyl; *i*, vascular system of root; *imd*, initiation of mucilage duct; *j*, sclerenchyma; *k*, root cap; *l*, leaf; *m*, meristematic plate; *md*, mucilage duct; *n*, medulla; *o*, indices of radial cell division in medulla; *p*, plumule; *ph*, phloem; *pph*, protophloem; *px*, protoxylem; *px′*, rupture of tissues caused by protoxylem; *q*, plerome; *r*, root; *s*, suspensor; *t*, leaf trace; *tr*, transfusion tissue; *u*, stem bundles; *v*, periblem; *w*, stipules; *x*, xylem; *y*, cork; *z*, anomalous thickening.

FIGS. 1–5.—Stages in intraseminal development showing single lateral cotyledon and gradual enlargement of its base to encompass axis. ×5.

FIG. 6.—Rupture of micropylar end of seed coat and protrusion of base of embryo. ×½.

FIG. 7.—Bending of base of embryo and exit of plumule. ×½.

FIG. 8.—Appearance of root. ×½.

FIGS. 9, 10.—Young seedlings. ×½.

FIG. 10*a*.—Longitudinal section through tip of unfolding leaf. ×5.

FIG. 11.—Dissection of aerial portion of two-year-old seedling. Nat. size.

FIG. 12.—Diagram showing spiral phyllotaxy of later leaves. Nat. size.

FIG. 13.—Transverse section of cotyledon. ×8.

FIG. 13*a*.—Transverse section of cotyledons of embryo developed on clinostat. ×8.

FIG. 14.—Transverse section of upper portion of cotyledon showing tendency to lobe. ×8.

FIG. 15.—Transverse section of same cotyledon nearer tip. ×8.

FIG. 16.—Diagram to show venation of cotyledon.

FIG 17.—Mesarch bundle from lower portion of cotyledon. ×380.

FIG. 18.—Exarch bundle from the middle region of cotyledon. ×380.

FIG. 19.—Longitudinal section of lower part of embryo emerging from seed. ×8.

FIG. 20.—Transverse section of the vascular plate of hypocotyl. ×225.

FIG. 21.—Diagram representing rise of cotyledonary bundles from vascular plate.

FIG. 22.—Diagram showing origin and course of first leaf traces.

FIG. 23.—Diagram showing origin and behavior of traces of second leaf.

FIG. 24.—Horizontal diagram of cotyledonary node.

FIG. 25.—Same node in plants developed on clinostat.

FIG. 26.—Transverse section slightly above cotyledonary node showing the four main cotyledonary bundles and the three groups of foliar bundles. ×60.

FIG. 27.—Longitudinal section of embryo somewhat less developed than the one represented in *fig. 19*. ×10.

FIG. 28.—Transverse section of stele of hypocotyl 2^{mm} above the section represented in *fig. 20*. ×225.

FIG. 29.—Transverse section of stele of hypocotyl immediately below cotyledonary node. ×225.

FIG. 30.—Transverse section of plant with median bundle of aborted cotyledon fairly well developed. Same level as *fig. 26*. ×8.

FIG. 31.—Section of same plant 140 μ above that represented in *fig. 30*. ×8.

FIG. 32.—Detail of portion of axis 0.2^{mm} below *fig. 30*, between the cotyledonary bundle *B* and the phellogen; shows group of cambium cells which gave rise to anomalous strand *z*. ×75.

FIG. 33.—Transverse section of the anomalous strand represented in base of cotyledon in *fig. 30*. ×135.

FIG. 34.—Cotyledonary node of plant with median bundle of aborted cotyledon well developed. ×150.

FIG. 35.—Stele of hypocotyl 0.36^{mm} below *fig. 34*; xylem and phloem collected in ring. ×150.

FIG. 36.—Section of stele of same plant 0.16^{mm} below *fig. 35*; shows breaking apart of phloem opposite the protoxylem. ×150.

FIG. 37.—Same, 0.2^{mm} below the previous section; separation of metaxylem. ×150.

FIG. 38.—Section of root stele between the third and fourth whorls of lateral roots. ×179.

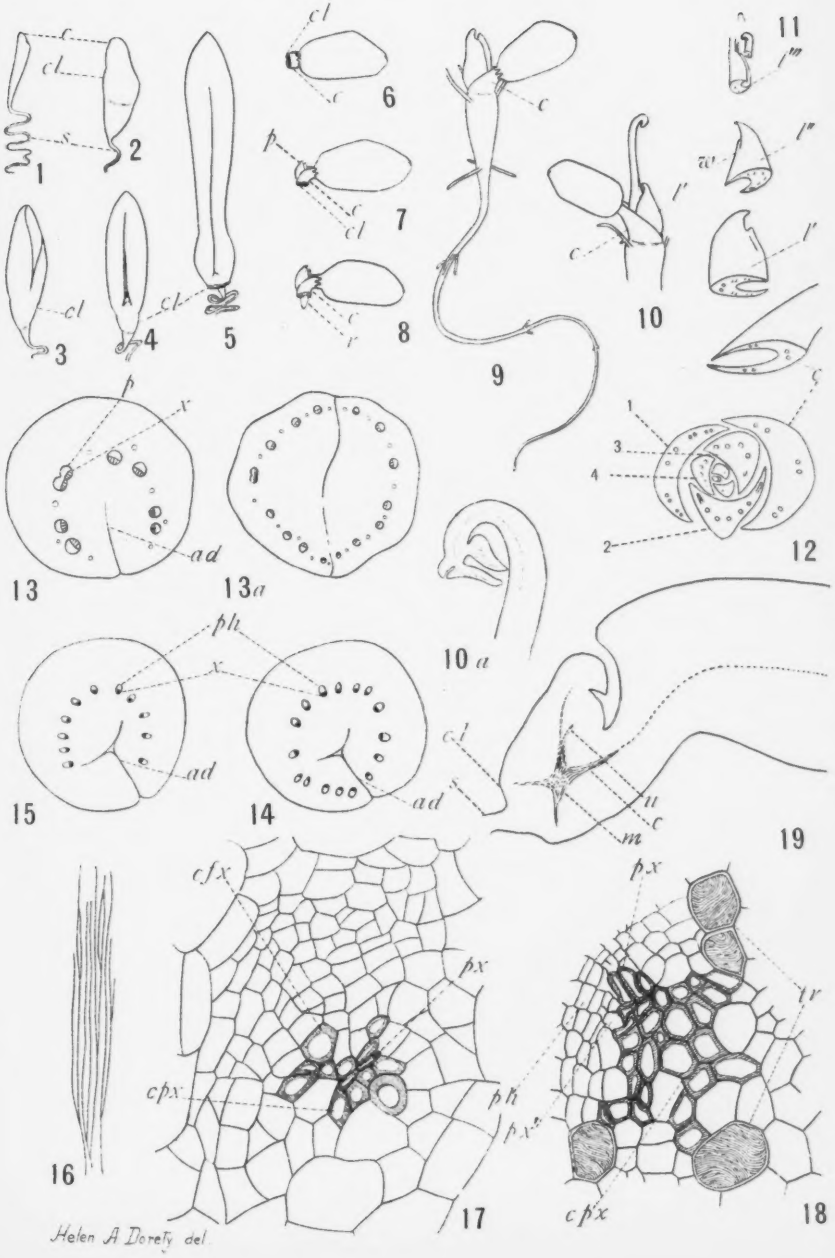
FIG. 39.—Section of stele near root tip at beginning of differentiation of xylem. ×225.

FIG. 40.—Transverse section of stele of lateral root showing exit of a tertiary root. ×225.

FIG. 41.—Longitudinal section of seedling showing beginning of root formation. ×10.

FIG. 42.—Detail of beginning of root formation. ×60.

FIG. 43.—Detail showing interruption between vascular tissues of hypocotyl and root. ×120.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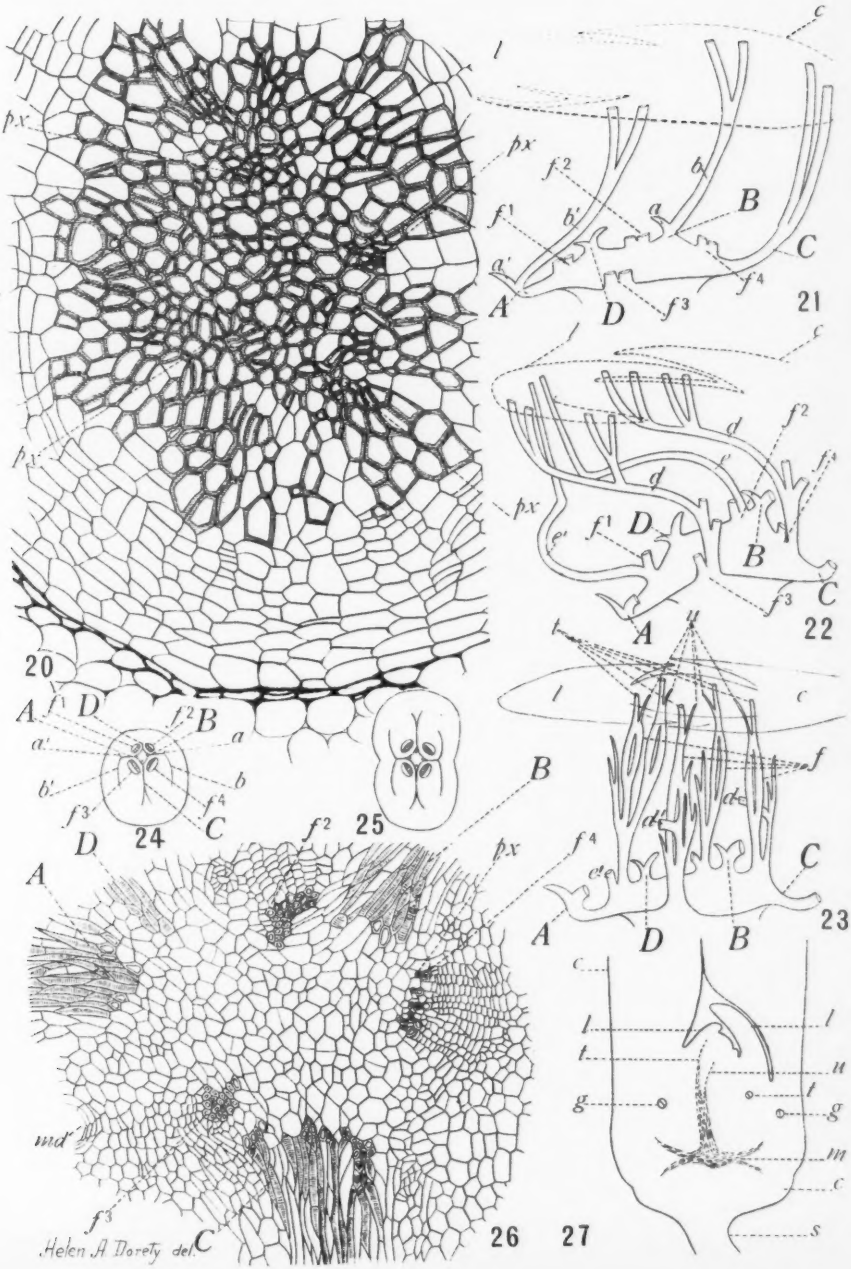
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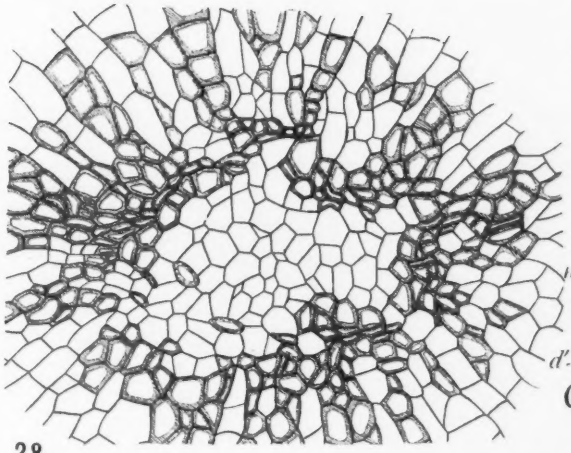
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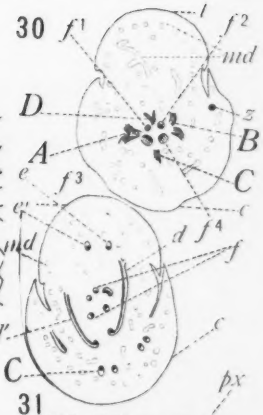
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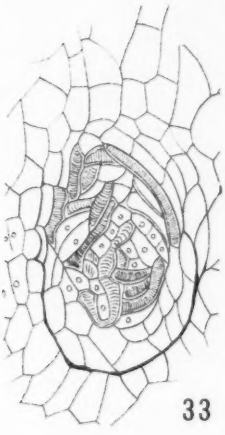
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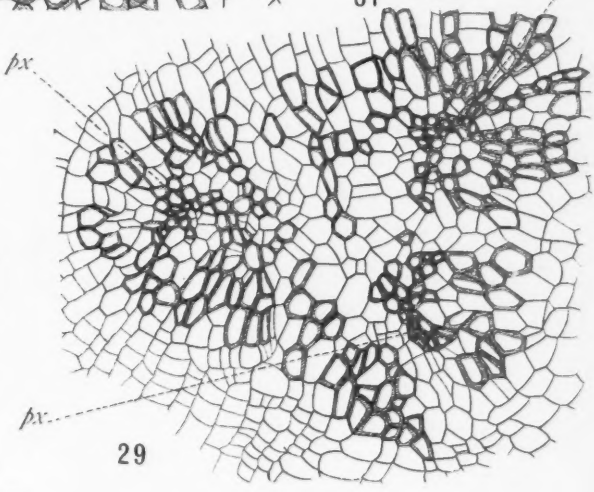
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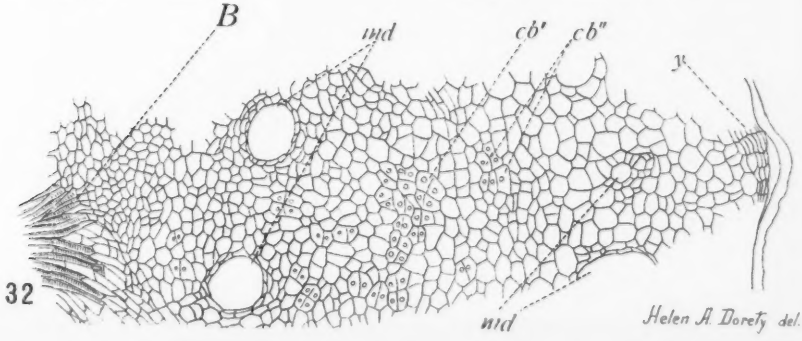
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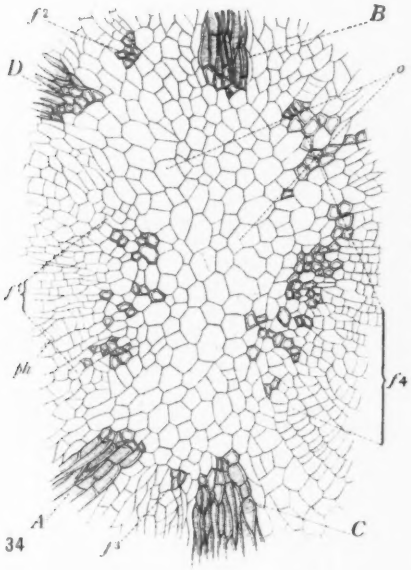

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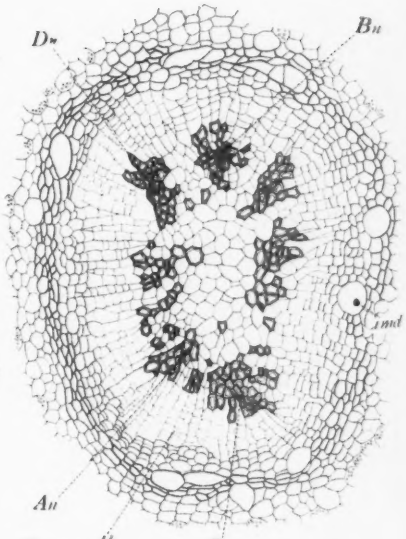

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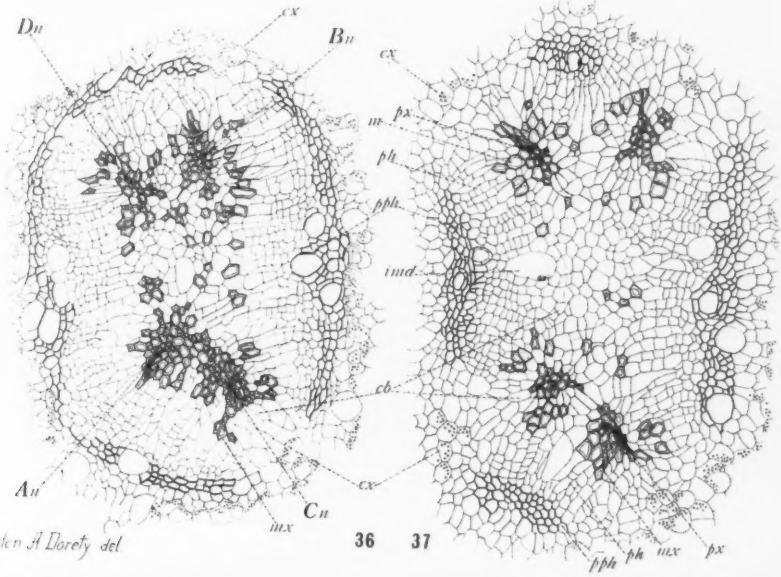

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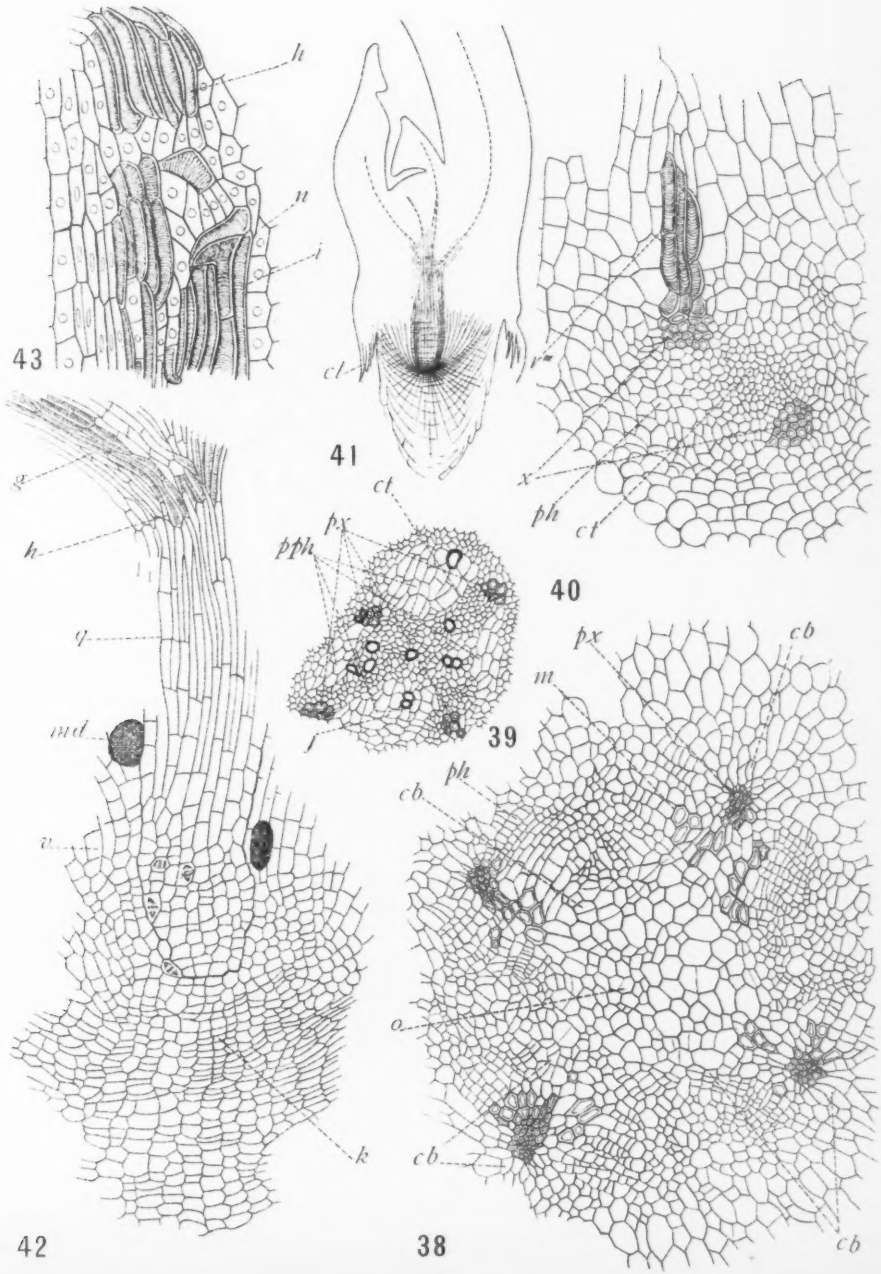
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BRIEFER ARTICLES

THE NUMBER AND SIZE OF THE STOMATA

For some educational purposes it is needful to know which plants possess the largest, the most numerous, the most readily observable, or the most definitely distributed stomata, and what quantities are involved in each of these features. The most important work upon the subject thus far is by WEISS,[1] but he includes only a few of those plants used for laboratory study in this country, namely, the common greenhouse plants and those readily raised in greenhouses from seed. Accordingly, in continuation of similar studies upon other topics as already described in this journal,[2] I have undertaken to obtain exact data upon this subject, with results recorded below. The study was begun, and carried well along, by Miss ALICE T. MITCHELL, while a senior student in Smith College, but she was unable to bring it into final form. I undertook at first simply to complete her work, but later I found it better, in order that all the results might represent a single method of treatment, to work over the entire subject from the beginning. The work has been done in the laboratory of plant physiology in Smith College, and has had the criticism and advice of Professor W. F. GANONG.

My method of study, in general, was that developed by LLOYD in his recent investigations[3] on the stomata of desert plants. I removed pieces of the epidermis from different parts of full-grown representative leaves, and dropped the pieces immediately into absolute alcohol. In order to test the possible effect of any shrinkage of the epidermis upon the numerical results, I made many comparisons of the data yielded by the epidermis alone with those yielded by untreated leaves; but I found no appreciable differences. I used throughout the same microscope and combination (Zeiss, objective DD, ocular 1 with cross hairs as an aid to counting). In counting the stomata, I adopted WEISS's method of counting all those falling within the field of vision, the area of which is easily calculated and reduced to square millimeters. For every species I counted the stomata, and calculated the mean, from thirty different fields selected at random from epidermis taken from three different plants (or, in two cases only, from different

[1] Jahrb. Wiss. Bot. 4:123, 196. 1865–1866.

[2] BOT. GAZETTE 40:302. 1905; 45:50. 1908; 45:254. 1908; 46:50. 1908.

[3] LLOYD, F. E., The physiology of stomata. Publ. Carnegie Institution. 1908.

shoots of the same plant), this precaution being observed in order to minimize any possible abnormalities of single plants.

In order to measure the size of the stomata, which involves the length and breadth of the guard cell apparatus when closed, and the length and breadth of the pore when most widely open, the same general method was used, except that, in order to prevent the closure of the pore through any possible wilting of the leaf, the epidermis was removed while the leaves were still attached to the plants. The pores of the stomata were found as a rule to be widest open at about 10 A. M., and accordingly the material was taken at that time. The measurements were made with an ocular micrometer, carefully valued by comparison with a stage micrometer. As in the case of the countings, thirty measurements were made upon material practically taken at random from three plants, and the figures in the table represent the mean of these. Only the linear dimensions of the pore are given in the table, but the area can very easily be determined by treating the opening as an ellipse,[4] though it is to be remembered, as BROWN and ESCOMB have shown,[5] that in the passage of gases through stomata, it is the linear dimensions, and not the area, which is important.

The data of the accompanying table may be summarized as follows. The stomata of our common greenhouse plants occur chiefly upon the under surfaces of the leaves, only about two-fifths (43 per cent.) having any upon the upper surface, and those almost invariably far less numerous than those upon the under. The most numerous stomata occur (in order of abundance) in Abutilon, *Ficus repens*, *Phaseolus vulgaris*, *Cucurbita Pepo*, and *Salvia involucrata*. The largest occur (in order of size) in *Triticum sativum*, Tulipa, *Avena sativa*, *Primula sinensis*, *Chrysanthemum frutescens*, and *Tradescantia zebrina*. In a general way, there is an inverse proportion between number and size of stomata. Taking all of the plants collectively, the number of stomata ranges from 0 through a mean of 121 to 484 per square millimeter, or, in general terms, they average over 100 to the square millimeter. The mean size of the open pores is $17.7 \times 6.7\ \mu$, and the mean area is 92 square μ. The total pore area for a square millimeter of leaf, therefore, is 11,132 square μ (121×92), which means that when the pores are open, one-ninetieth (or in round numbers over one-hundredth) of the epidermal surface is open.

Some other points worthy of mention in connection with the practical study of stomata in the laboratory are the following:

[4] Area of an ellipse $= \frac{\text{length}}{2} \times \frac{\text{breadth}}{2} \times \pi$.

[5] Nature 62:212.

The epidermis may readily be stripped in large pieces from the majority of greenhouse plants, notably from *Chrysanthemum frutescens, Cyclamen latifolium, Pelargonium zonale, Helianthus annuus,* Tulipa, *Vicia Faba,* and *Tradescantia zebrina.* It can be removed, though with difficulty, from some others, such as Abutilon, *Cestrum elegans,* and *Coleus Blumei,* while in some, e. g., *Ficus elastica* and *Hedera Helix,* it can be removed only by tangential sectioning with a razor.

STOMATA-QUANTITIES IN GREENHOUSE PLANTS

	Number. Minimum, mean, maximum in 1 square millimeter		Size. Length and breadth in microns			
			Upper surface		Lower surface	
	Upper surface	Lower surface	Guard cells closed	Pore open	Guard cells closed	Pore open
Abutilon	0	198–**333**–484	0	0	17×15	6× 3
Avena sativa	13–**25**–39	13– **23**–30	64×32	31× 7	70×36	38× 8
Begonia coccinea	0	22– **40**– 53	0	0	42×29	21× 8
Cestrum elegans	0	92–**146**–224	0	0	34×25	14× 7
Chrysanthemum frutescens	4–**15**–35	22– **34**– 61	57×31	31×11	58×31	33×11
Cineraria cruenta	0	39– **55**– 88	0	0	40×27	25× 8
Coleus Blumei	0	105–**141**–211	0	0	24×19	10× 5
Cucurbita Pepo	6–**28**–68	175–**269**–368	21×14	5× 2	20×16	6× 3
Cyclamen latifolium	0	44– **68**– 96	0	0	45×33	21× 7
Euphorbia pulcherrima	0	176–**233**–365	0	0	26×19	11× 7
Fagopyrum esculentum	17–**45**–66	127–**152**–184	27×20	10× 5	27×21	12× 6
Ficus elastica	0	83–**117**–180	0	0	40×36	19× 6
Ficus repens	0	228–**282**–365	0	0	21×17	5× 3
Fuchsia speciosa	0	52–**121**–193	0	0	39×28	19× 7
Hedera Helix	0	123–**158**–193	0	0	29×26	11× 4
Helianthus annuus	52–**85**–118	96–**156**–268	33×21	18× 8	36×21	22× 8
Heliotropium peruvianum	0	118–**149**–176	0	0	21×17	9× 5
Impatiens Sultani	0	118–**208**–396	0	0	26×18	9× 6
Lycopersicum esculentum	0–**12**–87	79–**130**–202	27×20	10× 5	33×23	13× 6
Oxalis Bowiei	0	52– **77**–118	0	0	28×21	11× 4
Pelargonium domesticum	8–**19**–35	39– **59**– 88	47×34	23× 8	45×32	24× 9
Pelargonium peltatum	4–**11**–22	13– **28**– 48	46×31	20× 6	45×30	22× 7
Pelargonium zonale	8–**22**–39	83–**118**–171	37×26	16× 9	37×25	19×12
Phaseolus vulgaris	13–**40**–96	184–**281**–356	25×14	8× 3	21×13	7× 3
Primula obconica	0	26– **47**– 70	0	0	39×34	17× 6
Primula sinensis	0	22– **31**– 48	0	0	59×46	30× 9
Ricinus communis	39–**64**–96	140–**176**–224	30×16	8× 4	30×20	10× 4
Salvia involucrata	0	211–**263**–330	0	0	20×16	9× 3
Senecio mikanioides	0	83–**114**–158	0	0	30×24	10× 7
Senecio Petasitis	0	66–**106**–149	0	0	44×32	23×10
Tradescantia zebrina	0	8– **14**– 22	0	0	55×33	31×12
Triticum sativum	22–**33**–44	8– **14**– 26	79×37	40× 7	84×35	38× 7
Tropaeolum majus	0	92–**130**–171	0	0	27×17	12× 6
Tulipa hybrid	22–**40**–57	35– **47**– 66	56×35	18× 7	72×35	32×10
Vicia Faba	35–**48**–66	39– **52**– 70	44×27	19× 8	46×28	20× 8
Vicia Faba equina	17–**34**–52	30– **48**– 61	42×26	18× 8	43×28	19× 8
Zea Mais	39–**52**–79	52– **68**– 88	47×36	19× 4	45×36	19× 5

Counting or measuring the stomata *in situ* on the leaf is possible with a few plants, notably *Begonia coccinea*, *Chrysanthemum frutescens*, *Fuchsia speciosa*, *Impatiens Sultani*, *Primula obconica*, *Pelargonium zonale*, *Tradescantia zebrina*, and *Vicia Faba*. In some others the condition of the pore can thus be observed, though the outlines of the guard cells are not clear: this is true in *Senecio Petasitis*, *Helianthus annuus*, *Cyclamen latifolium*, *Coleus Blumei*, *Cestrum elegans*, and *Phaseolus vulgaris*.

Marked variations in number and size of stomata occur, not only in different varieties of the same species, but in the same varieties grown under different external conditions. So far as my observation goes, however, the variation is greater in number than in size. Furthermore, while in most leaves the stomata are fairly evenly distributed over the surfaces containing them, in some, especially in oblong leaves (e. g., *Fuchsia speciosa*, *Helianthus annuus*, and *Impatiens Sultani*), the stomata are much more numerous near the base than near the tip (more than twice as many), and near the midrib than near the margin. For this reason very different figures might be given for the same leaf by different observers.

The opening and closing of the stomata of greenhouse plants is correlated closely with the time of day, and secondarily with the weather. As already noted, they are, as a rule, as wide open as they can be about 10 A. M. —this, of course, in well-watered plants. In favorable weather they remain wide open until about 2.30 P. M., when they begin to close, and they are mostly completely closed by 5 P. M., though some may remain open until 6. On hot days in the spring they may close as early as 12 M., probably because of incipient wilting of the leaf. If the stomata are closed by wilting, they may be made to open, partially at least, by immersion of the leaf in water.

The best plants for general laboratory study, taking account of ease of removing the epidermis, size and clearness of stomata, and commonness of occurrence in greenhouses, are, in order of excellence, *Chrysanthemum frutescens*, *Tradescantia zebrina*, *Pelargonium zonale*, *Fuchsia speciosa*, *Helianthus annuus*, and *Vicia Faba*.—SOPHIA H. ECKERSON, *Smith College, Northampton, Mass.*

THE ABSORPTIVE POWER OF A CULTIVATED SOIL

(WITH THREE FIGURES)

In the winter of 1908 we undertook a study of the absorptive power of a certain soil from one of the fields of the Michigan Agricultural College. In addition to the purely analytical methods which have been exclusively employed up to the present time in investigations of this kind, it was decided

to approach the problem in a somewhat different way. If the absorptive power of the soil is a factor of real importance, so far as plant life is concerned, it would be reasonable to expect that a concentration of a given salt solution which has proved to be detrimental for a given kind of plant when grown in soil extract would be beneficial or at least do no harm to the same kind of plant when grown in the soil itself.

With this idea in mind, we undertook (1) to determine the maximum tolerance of wheat seedlings to concentrations of certain salts when grown in soil extract to which known quantities of the salt in question were added; (2) to grow the same kind of plants in paraffined wire baskets,[1] using the

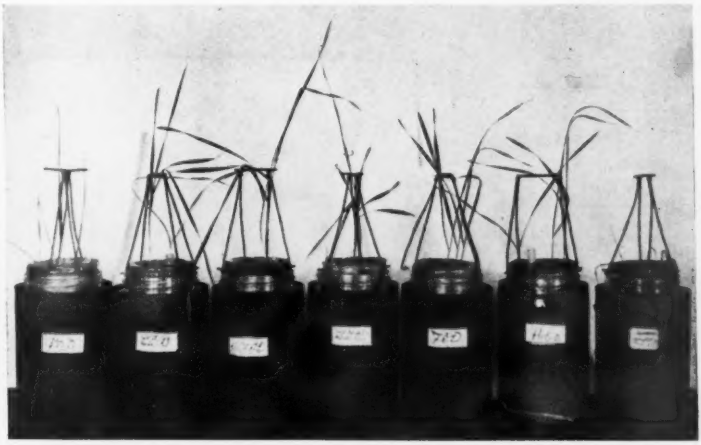

FIG. 1.—Tolerance of wheat seedlings to solutions of Na_2HPO_4; roots immersed in the solution: *1*, distilled water; *2*, soil extract (2:1); *3*, extract + 300ppm P_2O_5; *4*, same + 500ppm; *5*, same + 700ppm; *6*, same + 1000ppm; *7*, same + 2000ppm.

same kind of soil, making it up to the proper moisture content, and watering the plants with solutions of the same salts, of concentrations equal to and higher than the maximum tolerance determined for the extract by cultures.

Na_2HPO_4, KCl, and $NaNO_3$ were selected for use, separately and in various combinations. Some of the results obtained with the phosphate salt are given here. The extract was prepared by mixing a known quantity of air-dry soil with twice its weight of distilled water, leaving the mixture, with occasional stirring, for 48 hours, and filtering off the liquid. Portions

[1] The method devised by the Bureau of Soils, U. S. Dept. Agric. (see Bull. 23 and others of the Bureau).

of this extract were then made up to contain 300, 500, 700, 1000, and 2000 parts per million of P_2O_5 in the form of Na_2HPO_4.

The plants were set in small wire baskets (paraffined along the sides only) containing some sterile quartz sand, and the baskets placed in jars containing the proper extract, as shown in *figs. 1* and *2*. One set of plants was prepared with the roots immersed in the liquid, another with roots remaining in the sand The cultures were aerated by pouring the liquid from one jar into another and the losses by evaporation and transpiration were made up daily with the corresponding extracts. The effect is seen

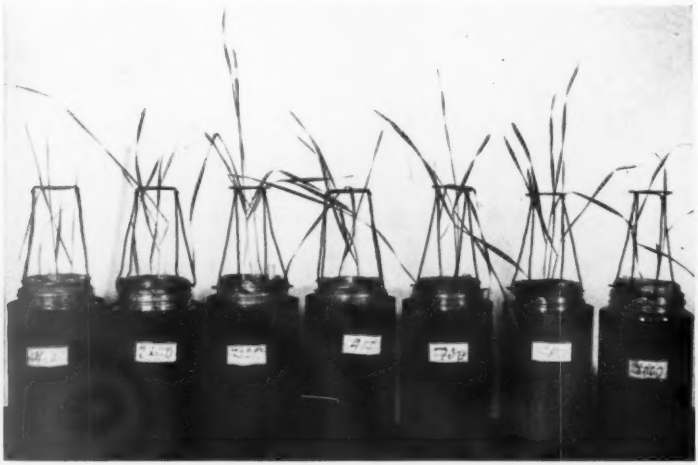

FIG. 2.—Tolerance of wheat seedlings to solutions of Na_2HPO_4 in soil extract; roots kept in sand of the baskets: *1*, distilled water; *2*, soil extract (2 water: 1 soil); *3*, extract + 100ppm P_2O_5; *4*, same + 500ppm; *5*, same + 700ppm; *6*, same + 1000ppm; *7*, same + 2000ppm.

from the photographs. Up to the 700ppm[2] concentration there was no marked difference between the two lots of plants. But while 700ppm immersed plants had a decidedly sickly appearance, the non-immersed ones looked quite healthy even in the 1000ppm extract. The 2000ppm of the non-immersed lot kept on struggling along even after three weeks from planting; while the immersed ones were killed in less than ten days.

It is thus seen that even the sterile quartz enables the plants to resist higher concentrations of the phosphates. The actual amount of salt

[2] Parts per million.

absorbed by the quartz was found to be about 10 per cent. in ppm of the original quantity. It was determined in the following way. The basket with the sand was removed from the jar. A sample was immediately taken to determine the water content; at the same time another portion of the same sand was washed with distilled water in ten successive portions. The washings were then evaporated to the volume calculated from the results obtained with the first sample (per centage of moisture in the sand) and the phosphates in the liquid determined. The phosphates in the extract contained in the jar from which the basket was removed were also determined,

FIG. 3.—Wheat seedlings in unmanured soil: upper row, left to right, all watered for last 7 days with P_2O_5 solution (*1*, 10,000^{ppm}; *2*, 7000^{ppm}; *3*, 6000^{ppm}); middle row, left to right, watered in same way (*1*, 5000^{ppm}; *2*, 4000^{ppm}; *3*, 3000^{ppm}); lower row, left to right, watered during all 29 days (*1*, 2000^{ppm}; *2*, 1000^{ppm}; *3*, distilled water).

and the difference between these determinations taken as the amount of phosphates absorbed by the sand.

The tolerance of the wheat seedlings to concentrations of the phosphate was very much increased when grown in the soil proper, as shown in *fig. 3*. Even concentrations of 6000^{ppm}, as seen also from the columns of "green" and "air-dry" weights in Table A, had no injurious effect on the plants. Only a concentration of 10,000^{ppm} was distinctly harmful.

The addition to the soil of unsterilized, fairly well-rotted cow manure,

in the ratio of 1:250, enabled the plants to resist still higher concentrations, as seen from the same table.

TABLE A

COMPARATIVE RESULTS AT THE END OF 30 DAYS

Treatment		Soil not manured				Manure not sterilized			
		No. of basket	Transpiration	Green weight	Air-dry weight	No. of basket	Transpiration	Green weight	Air-dry weight
During the entire 30 days	Dist. H_2O.....	27	224	3.00	0.41	16	220	3.5	0.48
	1000^{ppm} Na_2HPO_4	9	152	3.05	0.45	6	160	3.8	0.50
	2000 " "	12	146	3.00	0.48	18	143	4.0	0.60
During the last 7 days	*3000 " "	10	122	3.20	0.54	13	138	3.55	0.56
	4000 " "	11	135	3.10	0.42	17	143	3.5	0.58
	5000 " "	8	186	3.10	0.42	5	180	3.5	0.55
	6000 " "	7	178	3.20	0.41	4	187	3.8	0.58
	7000 " "	26	190	2.90	0.38	15	195	3.7	0.54
	10000 " "	25	180	2.20	0.34	14	184	3.5	0.52

* Up to the last 7 days the even numbers of the following set were watered with a 2000^{ppm} and the odd ones with a 1000^{ppm} solution.

That the increased tolerance of the wheat seedlings to the concentration of the salt is actually due, at least to a very great extent, to the absorptive power of the soil is sustained by the following facts:

(1) The analytical studies show that the soil actually removes large quantities of the phosphoric acid from the solution, as seen by the following table:

TABLE B

ABSORPTION OF PHOSPHATE BY THE SOIL

P.P.M. of P_2O_5 in original solution	Method	Soil not manured		Soil manured	
		Ppm. P_2O_5 in filtrate or percolate	Per cent. of P_2O_5 in Ppm. absorbed	Ppm. P_2O_5 in filtrate or percolate	Per cent. of P_2O_5 in Ppm. absorbed
1000	A*	795	20.5	745	25.5
1000	B†	728	27.2	698	30.2
2000	A*	1740	26.0	1700	30.0
2000	B†	1690	31.0	1674	32.6

* METHOD A.—25^{gm} of the soil mixed with 50^{cc} of the corresponding solution left with occasional shaking for 48 hours, filtered and the filtrate analyzed for phosphates.

† METHOD B.—200^{gm} of the soil placed in a wire basket (paraffined along the sides only) and 400^{cc} of the corresponding solution allowed to percolate through the soil; the percolate analyzed for phosphates.

(2) In the case of a nitrate salt, where the absorption is a negligible quantity, the tolerance of the immersed and non-immersed plants, as will

be more fully demonstrated in a later communication, is practically the same.

(3) The tolerance increases with the increase of the absorptive power; both factors being lower in the quartz, higher in the unmanured soil, and still higher in the manured soil.—JOSEPH ROSEN and CHARLES HELLER, *Agricultural College, Michigan.*

CURRENT LITERATURE

BOOK REVIEWS

Biologic types

In a recently published work Professor WARMING[1] has offered a valuable contribution to the knowledge of biologic types as these occur among plants. The subject is treated with rare skill, and although not a new one, the characterization of the types is carried out in such a way that many new points have been brought together and explained by the author in accordance with his own views, and embodying many of the most important results from his long experience.

The existence of biologic types was recognized many years ago. ALBERTUS MAGNUS and PORTA knew of them, but only vaguely. When therefore the author discusses the history of these types, he begins with ALEXANDER HUMBOLDT as the founder of plant-geography and physiognomy, so excellently outlined in his *Essai sur la geographie des plantes* and *Ideen zu einer Physiognomik der Gewächse*. It is interesting to follow the course of development of this science, so very greatly influenced by the Darwinian era. Since then we have witnessed the publication of an enormous literature dealing with biology, biologic types, and plant societies, all more or less combined under the modern term ecology.

A biologic type, according to the author, is the form which the vegetative organs have acquired in conformity with the surroundings; the particular structures, external and internal, which we find in stems and leaves, also in the complete shoot, but not in the flowers and fruits, which to a certain extent are independent of the surroundings. The vegetative organs owe their various modes of development and structure to climate and soil, as related to the associations known as hydrophytes, mesophytes, and xerophytes. As understood by WARMING the biologic types comprise two large groups, *heterophytes* and *autophytes*. The former of these include *holosaprophytes* and *parasites*. The autophytes are divided into five classes: aquatic plants, lichenoid plants, muscoid plants, lianas, and autonomic land plants. The sixth class comprises five subclasses: hapaxanthic plants, redivive herbs, rosette plants, creeping plants, and erect plants with long shoots persisting through many seasons.

The following examples may illustrate these subclasses. Among the hapaxanthic plants are those which are annual, biennial, and perennial, and which bloom only once. The redivive plants (*Stands* of the Germans) are those which die down to the ground at the end of the season, but which are provided with persistent subterranean organs (rhizomes, tubers, bulbs, etc.). The rosette plants form a familiar type, from the mountains especially; it may be dicyclic or pleiocyclic, and the leaves may be long and narrow, or roundish. In the creep-

[1] WARMING, E., Om Planterigets Livsformer. Copenhagen: G. E. C. Gad. 1908.

ing plants the organ that creeps may be either the stem, the stolons, or the roots. Finally, the fifth subclass contains species with the shoots elongated, which are able to persist for many years, for instance, the herbaceous *Arabis alpina*, also Armeria, Androsace, Eritrichium, etc.; and among the woody forms, Bambuseae, Coniferae, the dicotyledonous trees, etc.

An interesting chapter is devoted to the leaves and leafy shoots. The author recognizes three factors as being the direct cause of the shape of the leaf: (1) its function as an organ of assimilation; (2) the medium in which it lives (air or water); (3) the particular structure (outline especially) which belongs to the species, thus constituting a specific and very marked character developed through relationship. But the author makes no mention of the position of the leaf upon the shoot as having any bearing upon its form, although this seems an important factor when one remembers the great variation in leaves from seedling to mature plant, so profusely illustrated in North American plants especially.

The arrangement of plants according to their biologic character is a most difficult task, if really possible. To arrange plants in accordance with their vitality, as for example annuals, biennials, and perennials, gives no satisfaction; yet the question of age is of no small importance in classification. The characterization of biologic types, when comprising the structure of the shoot, leads us into perplexing difficulties, on account of the enormous number of intergrading forms that exist, of rhizomes for instance. It appears to the reviewer that the autonomic land plants have been classified less successfully than the others. However, we know of no system published, so far, where the classification of this particular group of plants has been outlined in any way clearer than the one suggested by WARMING. Much would be gained if the ecologists would follow his example and study the plants in the field, and not merely in the laboratory. It seems very strange that modern botanists pay so little attention to the study of organography, which actually is one of the bases of ecology; and plant-geography is indeed of no less importance. WARMING'S paper contains in itself an excellent guide to future workers in this line, and we hope that translations may follow soon, so as to make the paper more accessible to foreigners.—THEO. HOLM.

A text-book of botany and pharmacognosy

A second edition of KRAEMER'S *Text-book* has appeared,[2] designed primarily for students of pharmacy, for pharmacists, and for food and drug analysts. Perhaps it is not the province of a botanist to review it, but it certainly is an interesting illustration of the kind of botany required of students of pharmacy; besides, some of the introductory chapters deal with botany in the ordinary sense. The first impression is that of a mass of details, without any thread of continuity, which makes a book of reference rather than a textbook, a book to consult rather than to read.

[2] KRAEMER, HENRY, A text-book of botany and pharmacognosy. pp. vi+840. *pls. 321* (*figs. 1500*). Philadelphia and London: J. B. Lippincott Co. 1907.

The first chapter describes the "principal groups of plants," in which types are selected to represent the groups. The descriptions are systematic in form, encyclopedic in content, and entirely unrelated. Just what the student is expected to do with this part is not clear. There follow chapters on the "outer morphology of angiosperms" and "the inner morphology of the higher plants." This old breaking-up of a subject that cannot be broken is artificial in the highest degree. The confusion is increased by referring to the first topic as "the anatomy or outer structure of the angiosperms;" and to the second as "the inner structure or histology of the higher plants." This may be what students of pharmacy need, but it is not modern botany.

The remaining chapters deal with the professional details of pharmacy; although under Part I, which bears the title "Botany," there appear chapters on the "classification of angiosperms yielding vegetable drugs," and "cultivation of medicinal plants." Part II is entitled "Pharmacognosy" and contains chapters on crude drugs, powdered drugs, and foods. Part III is devoted to "reagents and microscopical technique."—J. M. C.

NOTES FOR STUDENTS

Synapsis.—GRÉGOIRE has published[3] an interpretation of synapsis opposing HERTWIG'S new theory and confirming his own earlier view. He states that the nucleus in synapsis passes through three principal states (*leptotènes*, *pachytènes*, *strepsitènes*), and that synapsis represents a primary state of heterotypic prophase. He says that cytologists diverge into two schools: one believing that the pre-reduction of chromosomes occurs in the zygonema stage, the bivalent chromosomes in the strepsinema stage representing the paired true chromosomes in heterotypic mitosis; the other believing that the pre-reduction is effected by a folding back, in the strepsinema stage, of a chromosome which is believed to be composed of two somatic chromosomes attached end to end. However, they all agree in considering synapsis as a stage in the development of the heterotypic chromosomes.

Taking WOLTERECK'S conception, R. HERTWIG proposed an entirely new interpretation of synapsis, deduced from his theory of nucleoplasmic relation. The gist of the theory is this: increase of protoplasm cannot continue without an intervention of nuclear division which may or may not be followed by cell division. After cell division, the increase of protoplasm and of nucleus cannot proceed equally, the former generally being ahead of the latter; so the nucleoplasmic quotient (K/P) tends to diminish. HERTWIG designates the state of equilibrium as "nucleoplasmic tension." This tension causes a sudden and considerable increase of the nucleus, which results in the increase of chromatin. The increase of protoplasm and consequent increase of chromatin can be brought back to equilibrium only by nuclear division. Applying the theory to the nuclear division in tetradogeneses (sporogenesis, spermatogenesis, and ovogenesis), he

[3] GRÉGOIRE, VICTOR, Les phénomènes de l'étape synaptique représentent-ils une caryocinèse avortée? La Cellule **25**:87–99. 1908.

found an objection, especially where a considerable increase of the ovocyte is not followed directly by nuclear division. For the sake of harmonizing the theory with the phenomena, he suggested that synapsis is really an abortive division. This theory of synapsis naturally tends to interpret synapsis not as a stage preparing for the heterotypic chromosomes, but only as an abortive form of nuclear division. The work of POPOFF on ovogenesis of Paludina is the material on which the theory is based. POPOFF apparently considers the bivalent chromosomes after synapsis and before the growth period in ovogenesis of Paludina as tetrads. These chromosomes completely disorganize in the diplonema stage, and the heterotypic chromosomes which appear after the growth period are a new formation, without any connection with the chromosomes that emerged from synapsis. Moreover, WASSILEIFF considers that in spermatogenesis of Blatta there occurs a pulverization of chromosomes during synapsis, and he believes that it is a trace of abortive nuclear division.

GRÉGOIRE remarks, under the headings spermatogenesis, sporogenesis, and ovogenesis, that such an interpretation cannot be in harmony with the vast majority of cases of synapsis, which are believed to be an important stage in the preparation of heterotypic chromosomes. In conclusion, he emphasizes synapsis as a fundamental stage, which constitutes a primary state of heterotypic prophase and not as an abortive kinesis.—SHIGÉO YAMANOUCHI.

Soil fertility.—The Bureau of Soils is doing an excellent work in seeking the explanation of the differences in productiveness of soils along the lines of a rational physiology. In spite of various attacks upon the principles which they are developing, the work commends itself to the unprejudiced as consonant with the modern phases of physics and physiology. Two recent bulletins contain valuable reports of research. CAMERON and GALLAGHER have shown[4] that when water has been added to a given soil in such proportion that it is in its most favorable condition for working and for plant development (as determined by expert gardeners), this is also the condition when to a pointed instrument it is physically most penetrable. This "optimum" water content varies with different soils from 4 per cent. (sandy) to 120 per cent. (muck). The apparent specific gravity or volume, the rate of evaporation, and some other physical features are also definitely related to the moisture content, changing in a marked way as the optimum water content is passed. It is also shown that the optimum moisture does not vary with the plant, but what is best for one plant is best for another in a given soil. Probably the penetrability of the soil is the important factor, since roots are thus able to reach their maximum development, and so to offer the largest possible surface for the admission of water.

Another bulletin, by GARDNER,[5] reports a vast number of experiments on

[4] CAMERON, F. K., and GALLAGHER, F. E., Moisture content and physical condition of soils. U. S. Dept. Agric., Bureau of Soils, Bull. 50. pp. 70. *figs.* 33. January 31, 1908.

[5] GARDNER, F. D., Fertility of soils as affected by manures. *Idem*, Bull. 48. pp. 59. *figs.* 5. March 21, 1908.

the effects of various fertilizers, including stable and green manures, upon wheat seedlings grown in pots. These were checked by field experiments, the results being mainly concordant. Here is presented the largest number of experiments yet made under uniform conditions, and while the conditions are still too complex for full analysis, the trend of the results is clear. Though in certain cases the composition of the soil as modified by the fertilizer is an important factor, it is rarely so important as the physical change. In very many cases, indeed, the crop yield can be as greatly increased by proper manipulation of the soil as by adding any sort of fertilizer. The experiments also indicate that the fertilizing of a particular field or region is a local problem, since even the same soil "types" from different localities show different results with the same fertilizer. (This may also be taken to indicate that the basis of classification of soils used by the Bureau is unnatural.)

Everyone who is interested in the growth of plants, either theoretically or practically, should read and reflect on these bulletins.—C. R. B.

Reduction and fertilization in Polytrichum.—The mosses have received practically no attention from cytologists. The small nuclei and some difficulties in technique are doubtless responsible for this neglect. A paper by the Drs. VAN LEEUWEN-REIJNVAAN[6] presents the results of an extended investigation of *Polytrichum piliferum*, *P. juniperinum*, *P. formosum*, and *P. commune*.

In spermatogenous tissue the nucleus contains a large deeply staining mass from which the chromosomes arise. From this mass there is cut off a small body which passes out of the nucleus into the cytoplasm and divides to form two centrosomes. These behave like typical centrosomes, and in the telophase are included within the nuclear membrane. At the last mitosis they remain in the cytoplasm and become blepharoplasts. At the same time a large piece of chromatin, which may be called a *Nebenkern*, is cut off and cast out into the cytoplasm, where it gradually degenerates.

In the sporogonium the mitoses show 12 chromosomes, 4 long, 4 short, and 4 medium. In the gametophyte there are 6 chromosomes, of which 2 are long, 2 short, and 2 medium. At the last spermatogenous division the 6 chromosomes unite in pairs, fusing longitudinally, so that one counts 3 chromosomes. Hence, the sperm contains 3 chromosomes, one long, one short, and one medium. At the division of the central cell of the archegonium, the ventral canal cell and egg cell each contain 3 chromosomes, one long, one short, and one medium. These two cells fuse with each other, and the egg, formed in this manner, is fertilized by two sperms. The fertilized egg contains 12 chromosomes, 3 from the egg proper, 3 from the ventral canal cell, and 3 from each of the two sperms. The

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[6] VAN LEEUWEN-REIJNVAAN, J. and W., Ueber eine zweifache Reduktion bei der Bildung der Geschlechtszellen und darauf folgende Befruchtung mittels zwei Spermatozoiden und über die Individualität der Chromosomen bei einigen Polytrichumarten. Recueil Trav. Bot. Neerl. **4**:(pp. 44. *pls.* 2). 1907.

writers believe that this behavior of the chromatin in Polytrichum supports the theory of the individuality of the chromosomes.

Commenting upon the above results from the standpoint of one not personally familiar with mitotic figures in mosses, abundant confirmation is needed before the account as a whole can be accepted. We are inclined to believe that the observations are largely correct and that the situation is extremely interesting, but that the final interpretation will not be so widely divergent from current notions of reduction and fertilization as the one proposed.—CHARLES J. CHAMBERLAIN.

Ginkgo.—Under the broad title *Ginkgo biloba* SPRECHER[7] gives a rather full account of the genus, arranged according to the following outline: embryo, young plant, leaf, secondary structure, flowers, pollen and fertilization, geographical distribution, uses and culture, fossils, and conclusions. Instead of giving a historical résumé followed by his own investigations, he has simply followed the above outline, using the available accounts and illustrations, and then filling in the gaps from his own investigations. With so large a subject and so many gaps to fill, an exhaustive investigation of any particular feature could hardly be expected. Most of the original work deals with floral development, leaf development, and anatomy. While the author has studied the gametophyte, it is in this field that he is most indebted to previous investigators. A large number of abnormalities in ovules, stamens, and sporophylls are recorded.

Of course there must be a guess at the phylogeny. While the sperms and certain characters of the ovules resemble those of cycads, in most respects Gingko is nearer the Taxaceae. Both Ginkgo and the Taxaceae have come from a Filicales stock which has given rise to the Cycadophytes and also to the Cordaitales and Ginkgoales, the point of departure being in the neighborhood of the fossil Botryopteridaceae.

The book will be useful for reference. It should be regarded as a compilation, supplemented by extensive personal observations, rather than as a work in which research is the predominant feature.—CHARLES J. CHAMBERLAIN.

SHAW[8] has investigated the vascular anatomy of the ovulate strobilus of Ginkgo, chiefly with reference to the morphological nature of the "collar." From aberrant material, which seems to appear abundantly enough under Japanese cultivation, it has been inferred that this collar is a much reduced megasporophyll. From this current view SHAW dissents, on the basis of testimony obtained from the vascular anatomy. The vascular tissue of the collar is "inverted," and a comparison with Lagenostoma shows a similar situation in that seed. The author therefore suggests that the collar of the Ginkgo ovule is a vestige of the well-developed cupule found investing the seeds of many of the Cycadofilicales. —J. M. C.

[7] SPRECHER, ANDREAS, Le *Ginkgo biloba* L. pp. 208. *figs. 225.* Genève. 1907.

[8] SHAW, F. J. F., A contribution to the anatomy of *Ginkgo biloba.* New Phytol. **7**:85–92. *figs. 16–18.* 1908.

Heliotropic tone.—PRINGSHEIM discusses in a long and rather technical paper the influence of illumination upon heliotropic tone.[9] By heliotropic tone he means that internal condition of responsiveness which determines the position of the cardinal points of reaction—the liminal and optimal illumination both for positive and negative response, and the indifferent zone. He shows that the reaction time of heliotropic plants diminishes with increasing intensity of light, rapidly at first, then more slowly and finally becomes constant. Beyond this a false optimum (really temporary indifference) is reached, but only in plants grown in the dark. If such plants, however, after being taken from the dark are rotated for a time in a light to which they will later react, the indifference disappears and the reaction, contrary to the earlier statements, is actually accelerated. This reaction, by a plant attuned to a certain light, is indeed the speediest possible at that intensity. In fact during the first part of the illumination of a plant of low heliotropic tone, the direction of the light is of no significance; for whether rotated or even illuminated from the opposite direction, it reacts just as quickly as though continuously illuminated from one side. The same is true in plants of high tone with stimuli of low intensity. During this first period the plant is merely adjusting its tone to the illumination. This alteration of tone is to be considered as an effect upon the excitable structure itself, produced either by a like or an unlike stimulus. One must distinguish between accommodation or adjustment to a given illumination and *Umschaltung* which determines whether the reaction is to be positive, negative, or none. This *Umschaltung* is dependent on the difference between the existing tone and that corresponding to the intensity of the illumination. If a low-toned plant be brightly lighted it reacts negatively; if weakly, the response is positive. If a high-toned plant is brightly illuminated, it reacts positively; with weak light, it does not respond at all until the tone has fallen far enough, when a positive reaction occurs. The tone in both cases follows the intensity of the illumination, but rises more quickly than it falls. All hypotheses which predicate heliotropic tone as a constant are faulty. The phenomena line up with those already known in certain other organisms and in the human retina, whence it seems probable that they are part of a general physiological law as to light perception.—C. R. B.

Geotropism and heliotropism.—The mutual effect of geotropic and heliotropic stimulation has been the subject of several papers, notably those by WIESNER, NOLL, and CZAPEK. VON GUTTENBERG, working in PFEFFER'S laboratory, has lately attacked the problem whether or not when they operate simultaneously on parallelotropic organs an alteration of geotropic tone occurs.[10] He concludes, contrary to other interpretations, that it does not, finding it possible by choosing

[9] PRINGSHEIM, ERNST, JR., Einfluss der Beleuchtung auf die heliotropische Stimmung. Beitr. Biol. Pfl. 9:263–306. 1907.

[10] VON GUTTENBERG, H. RITTER VON, Ueber das Zusammenwirken von Geotropismus und Heliotropismus in parallelotropen Pflanzenteilen. Jahrb. Wiss. Bot. 45:193–231. 1907.

an appropriate intensity of light in each case to balance the gravity stimulus without any effect on geotropic sensitiveness itself. Thus in the coleoptile of *Avena sativa* light of about 55 meter-candles, in the hypocotyls of *Brassica Napus*, *Lepidium sativum*, and *Agrostemma Githago* respectively 525, 666, 1026 m.-c., compensates gravity when each stimulus acts at 90°. When equivalent light acts at right angles to gravity (plants vertical, light horizontal) the parallelotropic organs take a resultant position, departing about 45° from the direction of each. On the elimination of the one-sided action of gravity by the clinostat, however, they become parallel to the light rays; but even in the final position of rest they have not lost their sensitiveness to gravity.

The geotropic series of reactions is quicker than the heliotropic, when the light is reduced to the compensating point; consequently, when light and gravity act antagonistically, the geotropic curvature appears first, and the maximum of heliotropic stimulation does not appear until much later. While these results are strictly true only for the plants observed, yet the principle is probably valid for others.—C. R. B.

Development of Juniperus.—Two preliminary accounts of fertilization in Juniperus were noted in this journal (**40**:318. 1907). The two accounts differed mainly in regard to time relations, NORÉN stating that the interval between pollination and fertilization was over a year, while SLUDSKY claimed that the development from megaspore to embryo occupies only a single summer. The present account[11] shows that NORÉN was right, SLUDSKY having made a mistake in estimating the age of the cones. The pollen grain in the uninucleate condition reaches the nucellus the middle of June and soon divides into a tube cell and generative cell, the latter remaining undivided until the following May, when it forms the stalk and body cells. Early in July the body cell gives rise to two equal male cells. In the nucellus there are several sporogenous cells, only one of which divides to form megaspores, the others becoming a nutritive jacket about the functioning megaspore. Usually only three cells of the tetrad are formed. In the archegonium there are four neck cells; and a ventral canal nucleus is formed, but never becomes separated from the egg by a wall. Fertilization occurs about the middle of July and the fusion nucleus passes to the bottom of the egg, where three mitoses give rise to eight free nuclei which become arranged in two zones. Walls now appear and the cells of the upper zone divide to form the rosette and suspensor.

The account is very full, cytological details of reduction and fertilization being figured and described.—CHARLES J. CHAMBERLAIN.

Hygroscopic movements of living leaves.—The leaves of some species of Rhododendron exhibit variation movements which follow the recurrence of freezing and thawing weather. The usual position of the leaves is horizontal, with the blade expanded. At freezing temperatures the edges of the leaves curl

[11] NORÉN, C. O., Zur Entwickelungsgeschichte des *Juniperus communis*. Upp-sala Universitets Ärsskrift **1907**:1–64. *pls. 4.*

under and the petioles allow drooping to occur. With the recurrence of thawing weather the blades expand and the leaf resumes its horizontal position. HANNIG[12] has found that the rolling of the leaf is due to a loss of imbibition water by the cell walls, and especially by the walls of the spongy parenchyma. The movements may be artificially induced by conditions which cause the cell walls to lose water and so allow a contraction of the walls to occur. The formation of ice, excessive transpiration, etc. are such conditions. The author is inclined to regard this as the first known instance of hygroscopic movements by living leaves. To the reviewer it seems that he has made a closer analysis of the cause of the movements, and his discovery consists in showing that while turgor variation is a prominent and accompanying feature, the real cause is the fluctuation in the content of imbibition water in the cell walls. It seems likely that many of the leaf movements which have hitherto been regarded as due to turgor changes may later be found to be caused by swelling and shrinkage of the cell walls. The author has not overlooked the fact that some leaves whose structure is apparently as well adapted to such movements as those of Rhododendron do not exhibit them.—RAYMOND H. POND.

Embryo sac of Nymphaea advena.—Miss SEATON[13] has examined the embryo sac of this species, giving an account of its earlier stages. Abundant material has enabled her to fill in some desirable details. The archesporium is distinguishable before the integuments begin to develop; and by division of the parietal cell and the epidermal cells the functioning megaspore becomes covered by a sterile nucellar cap six to ten cells deep. The sac develops a conspicuous tubular prolongation into the chalaza, and the fusion nucleus rests in the narrow connection between this chalazal haustorium and the broader micropylar portion of the sac. At the first division of this nucleus there is no wall (contrary to previous observation), and one of the daughter nuclei passes to the end of the chalazal tube. As before reported for the family, the proembryo is spherical and almost completely invested by endosperm. The monocotyledonous character of Nymphaeaceae is inferred, but no new evidence for it is advanced. This claim, which habitually accompanies the recent studies of Nymphaeaceae, is founded upon certain rigid preconceptions as to what constitutes a monocotyledon. It might be well for investigators of this group to try the effect of their work upon the rigidity of the old definitions.—J. M. C.

Araucarians of the Atlantic coastal plain.—BERRY[14] has called attention anew to the wide distribution of araucarians in the Mesozoic, especially as contrasted with their present very restricted range. A Mesozoic distribution of the

[12] HANNIG, E., Ueber hygroskopische Bewegungen lebender Blätter bei Eintritt von Frost und Tauwetter. Ber. Deutsch. Bot. Gesells. **26a**:151–166. 1908.

[13] SEATON, SARA, The development of the embryo sac of *Nymphaea advena*. Bull. Torr. Bot. Club **35**:283–289. *pls. 18, 19.* 1908.

[14] BERRY, EDWARD W., Some araucarian remains from the Atlantic coastal plain. Bull. Torr. Bot. Club **35**:249–260. *pls. 11–16.* 1908.

group from Greenland to Patagonia in the western hemisphere and from Spitzbergen to Cape Colony in the eastern is to be contrasted with its present occurrence in South America and the Australasian region. This means that araucarians have disappeared from North America, Europe, Africa, and practically all of Asia. Recent investigations in the Atlantic coastal plain show that the group not merely occurred in that region during the Mesozoic, but was abundant, perhaps the most abundant coniferous type of the older Mesozoic. From this region BERRY describes three new species: *Araucarites Zeilleri*, from New Jersey; *Araucaria bladenensis*, from North Carolina to Alabama; and *Araucaria Jeffreyi*, from North Carolina.—J. M. C.

Embryo sac and embryo of Urticaceae.—MODILEWSKY[15] has examined twelve genera of Urticaceae (Urtica, Elatostema, Laportea, Urera, Parietaria, Fleurya, Boehmeria, Dorstenia, Morus, Celtis, Cannabis, and Humulus), and finds that the embryo sac and embryo are in general of the ordinary dicotyledonous type. *Elatostema sessile*, *Dorstenia drakeana*, and *D. contrayerva* are said to be parthenogenetic, and *Celtis occidentalis* is chalazogamic. In the species of Dorstenia and in *Urtica cannabina* the antipodals multiply, but finally disappear. The polar nuclei fuse very early, and in Elatostema endosperm formation occurs without polar fusion. In *Urtica cannabina* a conspicuous antipodal haustorium is developed, and a much smaller one appears in *U. urens*. Many other details are recorded, but they are of no special significance.—J. M. C.

Black rot.—The black rot of the grape is the subject of a recent bulletin by REDDICK and WILSON,[16] which is mainly popular in nature, and is well illustrated and clear. The spores germinate on the vines only in the presence of water. Infection is noticeable after a period of twelve to twenty days, or upon the berry in eight to fourteen days. After discussing the control, it is stated that four acres, well sprayed, made a gain of 1662 pounds, equalling a saving of $32.95 per acre. It is recommended that mummied fruit be picked to avoid the spread of the disease, that the ground be turned over as completely as possible, to bury rotted berries, and that the vines be sprayed with Bordeaux mixture, as has been recommended heretofore.—F. L. STEVENS.

Leaves in autumn.—TSWETT summarizes the knowledge regarding the emptying of leaves in autumn thus.[17] It may be considered as settled that the nitrogenous compounds diminish and are carried back, proteolysis simplifying the proteins to this end; results as to phosphorus compounds are more contradict-

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[15] MODILEWSKY, JAKOB, Zur Samenentwicklung einigen Urticifloren. Flora **98**: 423–470. *figs.* 71. 1908.

[16] REDDICK, DONALD, and WILSON, C. S., The black rot of the grape, and its control. Cornell Univ. Agric. Exp. Sta., Bull. **253**:367–388. April 1908.

[17] TSWETT, M., Ueber die Verfärbung und die Entleerung des absterbenden Laubes. Ber. Deutsch. Bot. Gesells. **26a**:88–93. 1908.

ory, with the weight of evidence in favor of their recovery; the removal of salts needs thorough investigation.

As to the autumnal pigments, he reports[18] that the yellows are due to a new pigment or group of pigments, which he proposes to call autumnal xanthophyll. He regards it as probably a decomposition product of the "normal xanthophylls, perhaps also of the carotin."—C. R. B.

Torreya in the Cretaceous.—BERRY[19] has described a new species of Torreya (*Tumion carolinianum*) from the Cretaceous of North Carolina, based on leaf-bearing branches, the leaves showing the distribution and character of the stomata. The genus exists today as isolated species, which are widely separated geographically, and this fact alone would suggest an ancient type. The discovery of intermediate stations will bring a knowledge of the time of general distribution and help settle the question of relative antiquity.—J. M. C.

Phylogeny of pteridophytes.—Lady ISABEL BROWNE[20] has begun a series of papers intended to bring together the large volume of recent work on the vascular anatomy of pteridophytes, and apply it to a consideration of the phylogeny and interrelationships of the group. This is a very useful service, for it organizes the scattered facts in convenient form, whether one accepts all the inferences or not. In the first two papers, the Sphenophyllales and Equisetales are presented and Lycepodiales begun.—J. M. C.

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[18] TSWETT, M., Ueber das Pigment des herbstlich vergilbten Laubes. Ber. Deutsch. Bot. Gesells. **26a**:94–101. 1908.

[19] BERRY, EDWARD W., A mid-Cretaceous species of Torreya. Am. Jour. Sci. **25**:382–386. 1908.

[20] BROWNE, ISABEL, The phylogeny and interrelationships of the Pteridophyta. A critical résumé. New Phytol. **7**:93–113, 150–166. 1908.